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(54) Title: RECOMBINANT HIV AND MODIFIED PACKAGING CELLS AND METHOD FOR TREATING ACQUIRED IMMUNE DEFICIENCY SYNDROME

(57) Abstract

A recombinant human immunodeficiency virus (rHIV) and recombinant mammalian cell-line (packaging systems) and a method for treating infection of cells by human immunodeficiency virus HIV. rHIV, such as rHIV-1, comprises a gene construction which includes a foreign gene. The expression of this gene is activated in human cells in the presence of wild-type HIV. This gene product can cause cell death in the presence of an appropriate drug, e.g. Acyclovir. This gene product is typically a viral thymidine kinase. rHIV is so constructed that is unable to replicate by itself due to the absence of a regulatory gene that is necessary for its replication, such as tat or rev or both. The recombinant mammalian cell-line packaging system comprises in its genome a recombinant gene construction which typically includes a functional regulatory gene from HIV which is missing from rHIV, such as the tat or rev genes or both. The method for treating infection of cells by HIV comprises administering a composition comprising rHIV, which is produced *in vitro* from a recombinant mammalian cell-line, and treating with a nucleoside analog, such as Acyclovir or Gancyclovir.

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RECOMBINANT HIV AND MODIFIED PACKAGING CELLS AND METHOD FOR TREATING ACQUIRED IMMUNE DEFICIENCY SYNDROME

FIELD OF THE INVENTION

The present invention relates generally to genetic engineering and also to gene therapy wherein a genetically engineered gene is included into a vector for the purposes of in vivo targeted therapy.

BACKGROUND OF THE INVENTION

The availability of genetic engineering, particularly in the form of recombinant DNA technology has increased the possibility of successful gene therapy. A particular desired scenario for gene therapy involved in vivo targeted therapy which is specific for the disease to be treated. Typically, a gene, which expresses a product that is useful in the treatment of the disease, is inserted into a vector, such as a virus, which is then administered to the patient suffering from the disease. There are numerous proposals in the literature for such treatments and vectors.

It will be appreciated that in recent times, considerable effort and resources have been devoted to treating one particular disease in humans which is usually fatal. This disease, acquired immune deficiency syndrome (AIDS) is caused by a particular retrovirus known as human immunodeficiency virus (HIV) which includes the virus known as HIV-1. Numerous

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treatments for this disease have been proposed and tested and some are currently in use. For example, AIDS is commonly treated by administering the drug AZT to AIDS patients. Moreover, ribozyme and anti-sense technology are currently being developed as possible treatments for humans suffering from infection by HIV. Many of these new technologies are discussed in volume 260 of SCIENCE, 28 May 1993 issue. As illustrated on page 1257 of this issue of SCIENCE, numerous therapeutics have been identified for various steps in the stages of the replication of HIV. The replication of HIV is attached by these proposed therapeutics in order to treat the disease. However, it has not been possible to cure the infected individual because of the ability of HIV to remain integrated into the cellular genome and also due to the frequency of changes that appears in the viral genome in every replication cycle.

Often, these various treatments are not permanent and are not economical, and they are often not specific enough. The lack of specificity of the treatments tends to cause complications arising from the effect of the therapeutic drug on uninfected human cells in the body of the patient being treated.

The present invention seeks to provide a novel treatment for AIDS as well as a genetically engineered HIV and a genetically engineered cell line for producing the genetically engineered HIV.

SUMMARY OF THE INVENTION

The invention provides a recombinant human immunodeficiency virus (r HIV) and a recombinant (modified) T-cell line having a functional regulatory gene from HIV, and a method for treating the acquired immune deficiency syndrome in a human caused by HIV.

The modified human immuno deficiency virus (rHIV) includes in its genome a modification (e.g. a deletion) of one of the HIV regulatory genes and the inclusion of a foreign gene. In one embodiment, the foreign gene is a viral kinase enzyme such as a viral thymidine kinase (tk) from the Herpes simplex virus (HSV-1). Typically, the rHIV is unable to express at lease one functional regulatory gene product of the genome of HIV because the genome of rHIV has been modified to either remove or incapacitate the gene which encodes this functional regulatory gene product. In one embodiment, this functional regulatory gene product may be either the tat protein or the rev protein of HIV.

The present invention also provides a modified mammalian cell line, such as a modified T-cell line, having in its genome a recombinant gene construction including a gene from the genome of HIV (usually the wild-type HIV-1). This foreign gene will typically encode a functional regulatory gene product of HIV. Typically, this functional regulatory gene product of HIV is the same functional regulatory gene product which the rHIV cannot produce or express. In this fashion, the modified T-cell line will support replication of rHIV while a normal T-cell line (or a normal in vivo T-cell) will not support replication of

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rHIV. In one embodiment, the modified T-cell line includes the tat gene from wild-type HIV (e.g. HIV-1) and expresses the gene product of this gene so that is becomes possible to replicate rHIV (tat-) in this cell line. This in vitro rHIV production is referred to as a packaging system.

The invention also provides a method for treating AIDS in a human by administering a composition which includes rHIV followed by administering a nucleoside analog. In a typical embodiment, the nucleoside analog is Acyclovir or Gancyclovir. In a typical implementation of this method, the rHIV is harvested from the modified T-cell line. The harvested rHIV will then be administered to the patient suffering from AIDS. rHIV will infect all HIV-infectable cells. In one embodiment, the tk gene included in rHIV will be actively expressed only in the presence of wild-type HIV. Treatment with Acyclovir completes this modality.

It will be appreciated that in the preferred embodiment rHIV (typically, rHIV-1) is incapable of replication on its own in a normal T-cell line or a normal T-cell. That is, it requires the modified T-cell line or T-cell having the regulatory gene which is depleted from rHIV-1. It will also be appreciated that rHIV produced according to the invention is highly infectious and has exactly the same host range as wild-type HIV-1. rHIV-1 is capable of super infecting human T-cells previously infected with wild-type HIV-1. Although rHIV-1 is incapable of replicating by itself in a normal cell, it will infect a previously HIV-1 infected cell as well as an HIV-1 infectable cell, but

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rHIV-1 will not replicate in cells that lack wild-type HIV-1.

In the case of double infection of a cell with HIV-1 and rHIV-1, rHIV-1 is stimulated to replicate and viral thymidine kinase is produced. This enzyme will phosphorylate a nucleoside analog, such as Acyclovir or Gancyclovir, which is a cytotoxic substance that will kill the HIV-1 infected cell. It will be appreciated that normal cellular thymidine kinase phosphorylates the nucleoside analog very minimally with no consequences. Thus, administering these nucleoside analogs is safe and approved (as these analogs have been successfully used to treat HSV-1 infections in humans); that is these drugs have been well tested due to the fact that they have been prescribed for the treatment of herpes viruses. If HIV-1 infects cells previously infected with rHIV-1, viral thymidine kinase is produced and upon treatment with Acyclovir or Gancyclovir or other nucleoside analogs, these dully infected cells would be killed. The problem of developing resistance to these compositions such as Acyclovir or Gancyclovir does not arise because (1) the time for developing a modified tk gene is not available, and (2) fresh inoculation of rHIV can always be administered to circumvent the problem.

It will be appreciated that repeated treatment may be needed to eradicate the HIV infection from infected individuals and prevent future reinfection from within. This direct in vivo gene therapy not only avoids the costs and complications of ex-vivo approaches but has the potential to become a simple out-patient office procedure. The treated patient can go on to other

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remedial therapies for the correction of other deficits.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be described while referring to the following figures which are presented for purposes of illustration:

Figure 1 shows a general method of producing a modified mammalian cell or cell line according to the present invention.

Figures 2a, 2b, 2c and 2d show various maps which illustrate the construction of the plasmid that is used to produce a modified mammalian cell or cell line according to the present invention.

Figure 3 shows a general method for producing a recombinant HIV and relevant control constructs according to the present invention.

Figure 4 shows a particular plasmid which is a gene construct containing proviral HIV-1.

Figure 5 shows a design strategy for a particular embodiment of the present invention for inactivating a particular regulatory gene of HIV-1.

Figure 6 shows a design strategy for creating a gene construct for rHIV-1 for a particular embodiment of the present invention.

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Figure 7 shows a particular gene construct for a particular embodiment of the present invention; this gene construct contains a foreign gene which is to be inserted into HIV-1 in order to create rHIV-1.

Figure 8 shows a general method for treating HIV infection with a recombinant HIV, as well as a method for producing the recombinant HIV.

Figure 9 illustrates the nucleoside analog Acyclovir.

Chart 1 shows a test for the replication of progeny virus from a T cell line transfected with various gene constructs.

Chart 2 shows results from the infection of mononuclear peripheral blood cells from four normal donors by the progeny virus produced by the modified T-cell lines of the present invention.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

While the present invention will be described by referring to specific examples of gene constructs, nucleoside analogs, foreign genes, and particular cell lines, as well as other details, it will be appreciated that this description is not by construed in a manner to limit the scope and spirit of the present invention. Moreover, numerous specific details which are well understood by those in art are described briefly so that the present invention is not unnecessarily obscured.

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The modified mammalian cell line, such as a modified human T-cell line having in its genome a regulatory gene from wild-type HIV, is created, in one embodiment, according to the general method shown in figure 1. In a typical embodiment, the modified cell line will be a human T-cell which is transfected with a plasmid containing an HIV-1 regulatory gene, such as the tat gene, thereby producing a modified T-cell line having the tat gene in its genome. According to the general method shown in figure 1, the HIV-1 tat/rev mRNA, labeled 101, is reverse transcribed in order to produce tat/rev cDNA, which is cut with restriction enzymes SalI and BamHI to isolate the two coding exons of HIV-1 tat cDNA, and this product is labelled as 102 in Figure 1. This tat cDNA is then inserted into a vector 103, typically downstream from a promoter region 104 to produce a resulting plasmid 105 as shown in figure 1. This plasmid is then used to transfect human T or B cells (e.g. the Jurkat T cell line) to produce a modified T-cell or a modified B-cell, such as T-cell 107 or B-cell 108. It will be appreciated that other regulatory genes of HIV, such as rev may also be inserted into a plasmid along with the tat gene and this plasmid may then be used to transfect T-cells or B-cells to thereby produce a modified T-cell or B-cell. These cells are typically immortalized cell lines capable of indefinite replication and thus are useful as packaging systems to prepare large numbers of the rHIV-1 described below.

A specific example of this general method shown in Figure 1 will now be provided in conjunction with Figures 2a, 2b, 2c and 2d. Figure 2a shows a map on the proviral DNA of the genes encoding for the HIV-1

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proteins. The two coding exons of the tat gene are indicated by arrows on Figure 2a. Figure 2b shows the splicing pattern for the tat/rev mRNA; the SalI and BamHI splicing sites corresponding to the tat cDNA (0.3 kb long) fragment 102 are indicated on the outline of its mRNA shown in Figure 2b. It will be appreciated that the gaps in the mRNA of Figure 2b are joined after conventional mRNA post-transcriptional processing. The resulting mRNA tat/rev is shown as fragment 101 in Figure 1, and this fragment is reverse transcribed as indicated above and then the cDNA is digested with SalI and BamHI to produce the cDNA tat fragment 102, which is 356 base pairs (bp) long and is isolated using conventional techniques.

The expression vector 103, shown in Figure 1 and Figure 2c, contains the origin of replication and the early region of the human papovavirus BK (allowing its amplification in human cells), a "cassette" of SV40 early prometer 104, splicing and polyadenylation sequences that permit expression of a cDNA, and plasmid sequences from pBR322. The expression vector 103 also includes the bacterial gene for the aminoglycoside phosphotransferase (Neo) under the control of the SV40 early region promoter and polyadenylation sequences, which confers resistance to the aminoglycoside antibiotic G418 when expressed in euharyotic cells.

The plasmid 105, shown in Figure 2d, contains the cDNA of HIV-1 tat gene and was derived from plasmid 103, shown in Figure 2c, in the following manner. The SalI-BamHI fragment 102 of 356 bp (which contains the two coding exons of the HIV-1 tat cDNA) was filled in using the Klenow enzyme (large fragment of DNA

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polymerase I) and inserted by conventional blunt end ligation into the unique XhoI site of vector 103 which was previously filled in with the Klenow enzyme. This blunt end ligation of the filled in fragment 902 into the vector 103 places the tat cDNA 102 fragment between the SV40 early promoter 104 and the SV40 splicing and polyadenylation signals (shown as SV40 s.p.s. in Figure 2d).

The Jurkat T-cell is derived from a human T-cell lymphoma and is used in an embodiment where T cells are transfected with the plasmid 105. This transfection produces a modified mammalian T-cell lone having tat+ (that is, having the tat gene which expresses functional tat protein). Plasmid 105 was introduced into these Jurkat T cells by electroporation using conventional electroporators (e.g. from Invitrogen Corporation). In one embodiment, a sample of 10^7 cells was suspended in 1ml of the media RPMI 1640 (which is chilled on ice to roughly about 0°C). Plasmid DNA (in the form of Plasmid 105) was added at concentrations of 100ng to 1 microgram per sample. Electroporation in a 0.4cm wide cuvette was carried out at 280mV and 960μF. Transfected cells were selected using the antibiotic geneticin (G418). Viable cells were recovered and expanded into cell lines which stably produce the HIV-1 tat protein.

The modified human immunodeficiency virus (e.g. rHIV-1) is produced according to the general method shown in figure 3. This method begins with a proviral HIV-1 DNA, labeled 201, which is inserted, using conventional recombinant DNA technology, into a plasmid to create plasmid 203. It will be appreciated that

this proviral HIV-1 DNA in one embodiment was constructed from two wild-type HIV and accordingly has the necessary regulatory genes which are essential for its replication. This is not only a replication competent virus, it is also highly infectious. Accordingly, the tat gene will be present in plasmid 203. The plasmid 203 may then be replicated in culture (e.g. in a bacterial culture) to produce a large number of such plasmids using conventional culturing technology. Some of these plasmids may then be harvested for the knock out or removal operation shown in figure 3 which creates plasmid 205 from plasmid 203, and similarly some of these plasmids 203 may be harvested for an insertion operation where in the tk gene 207 is inserted into the plasmid 203 to produce the plasmid 210. In an analogous fashion, the plasmid 205 is used to insert the tk gene 207 to produce the plasmid 209. A specific example is given below for incapacitating the tat gene in order to produce the plasmid 205 from the plasmid 203. It will be appreciated by those in the art that the tat gene or other essential regulatory genes may be removed or knocked out in order to render the gene incapable of expressing a functional regulatory gene product. For example, the rev gene bay be knocked out in addition to the removal or knock out of the tat gene.

After producing plasmids 210 and 209 from plasmids 203 and 205 respectively, conventional transfection operations are performed in order to introduce the respective plasmid into normal T-cell lines or modified T-cell lines. Alternatively, transfection operations may be performed on normal B-cell lines or modified B-cell lines. Currently, transfection of modified T-cell

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lines containing the tat gene of HIV-1 (wild-type) is preferred. Given this preference for transfection into T-cell or T-cell lines, this transfection procedure may be carried out as described above. Typically, the electroporation technique is used to cause transfection in order to efficiently introduce the DNA construct into the packaging cells. However, alternative methods of transfection may also be performed. In one embodiment a DNA construct, such as plasmid 209, may be transfected into T-cells (e.g. modified Jurkat tat+ cells) by first cleaving the construct into two pieces (e.g. including a cleavage at the SalI site) and then sequentially introducing (by for example electroporation) one piece into the T-cells and then the other piece into the same T-cells. Then, the T-cells may assemble the pieces to produce a competent cell packaging system (e.g. T-cells 211) which will produce the rHIV of the present invention.

In the case of the plasmid 210 which contains a complete copy of the HIV-1 genome as well as a complete copy of the tk gene (from, for example, HSV-1), this plasmid is transfected into a normal (tat-) T-cell or T-cell line, thereby producing a T-cell 212 having an integrated proviral HIV-1 genome which includes the tk gene from HSV-1. Transfection of a modified (tat+) T-cell or modified T-cell line which contains the tat gene from HIV-1 (Wild-type) with plasmid 210 produces a T-cell 214 which contains a integrated proviral copy of the HIV-1 genome with the tk gene as well as a further copy of the tat gene in the genome of the T-cell. It will be appreciated that these modified T-cells are produced according to the method described in conjunction with figure 1 above. It will be

appreciated that T-cells 212 and T-cells 214 are used as controls in the experiments described below which establish the efficacy of the methods for producing the proper and desired gene constructs and the desired rHIV-1 and modified T-cells/T-cell lines.

Plasmid 209 is also used to transfect both normal T-cells or T-cell lines and modified T-cells or modified T-cell lines. Plasmid 209 contains a full copy of the HIV-1 genome with the exception that a regulatory gene has been disabled or totally removed (e.g. tat-), and it also includes a copy of the tk gene (full functional copy capable of expressing viral thymidine kinase). In the embodiment shown in figure 3 the functional regulatory gene of HIV which has been disabled is the tat gene, and thus the plasmid 209 is labeled as "tat-" to indicate the absence of this functional regulatory gene of HIV-1. After producing many copies of the plasmid 209 and harvesting these plasmids from their bacterial host, one sample of plasmids 209 is used to transfect a normal (tat-) T-cell or T-cell line, and another sample of plasmid 209 is used to transfect a modified (tat+) T-cell or T-cell line, thereby producing T-cell 213 or T-cell 211 respectively. The transfection procedures are similar to those described above. The T-cell 213 will be incapable of producing complete HIV-1 viral particles due to the absence of the tat gene on both the genome of the T-cell and the proviral copy of HIV-1 in the genome of the T-cell. These T-cells, such as T-cell 213 may be used as a control in the experiments shown below to establish the efficacy and functionality of rHIV-1 as well as the modified T-cell line. The T-cell 211 will be able to produce multiple viral products, in

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this case the modified or recombinant human immunodeficiency virus referred to as rHIV-1 which lacks the functional regulatory gene tat and consequently is unable to express the gene product of this gene but does include a copy of the HSV-1 thymidine kinase gene. This viral product may then be used to treat AIDS in the method described below. The T-cell 211 is capable of producing this recombinant virus by virtue of having a separate copy of the tat gene which is expressed by the transcription and translation system of the modified T-cell. That tat gene product expressed in this T-cell 211 allows the proviral HIV-1 having the tk gene (tk+) but not having a functional copy of the regulatory gene tat (tat-) to replicate itself to produce multiple copies of rHIV-1 (tat-, tk+). Thus, T cell 211 may be considered a packaging system for producing this recombinant virus.

It will be appreciated that numerous gene constructs such as the plasmids 203, 205, 209, and 210 may be constructed in various ways to achieve the results described herein. Thus, the specific examples given below for these particular gene constructs are merely one implementation of the present invention. In a particular embodiment of the present invention, plasmid 203 is pNL 43, shown in figure 4. In this same embodiment, plasmid 205 is plasmid pNL43dBM, shown partially in figure 5, which shows a design strategy for the inactivation of the HIV-1 tat gene. The tk gene insert 207 shown in figure 3 is produced in this same embodiment according to the design procedure shown in figure 7. The plasmid 209 in this same embodiment, which is the plasmid of interest since it produces rHIV-1 in the modified T-cell line, is the plasmid

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pNL43dBMtk and is produced according to the design procedure shown in Figure 6. The control plasmid 210 in this embodiment is known as plasmid pNL43tk.

After producing multiple copies of the recombinant HIV, which in the particular embodiment described above is rHIV-1 (tat-, tk+), the treatment of humans infected with HIV-1 may then be performed by administering the recombinant virus and a nucleoside analog to the same patient. This will be described by referring to figure 8 which also provides an overview of the invention. While figure 8 presents one example of the method according to the present invention, it will be appreciated that certain of the steps (e.g. 801, 803, 805, and 807) may be performed in a sequence which is different from that shown in figure 8. The method begins in step 801 wherein a T-cell line (or a B-cell line) is transfected with a plasmid which contains the functional HIV regulatory gene (e.g. tat gene) in order to produce a modified T-cell line which will be the packaging system used to produce the recombinant in HIV of the present invention. In step 803, a proviral HIV construct is created without the functional HIV regulatory gene (e.g. tat gene) which has been inserted into the modified T-cell in step 801. Then in step 805, a foreign gene such as the HSV-1 thymidine kinase gene is inserted into the proviral HIV construct created in step 803. This creates, for example, rHIV-1 (tat-, tk+). Then in step 807, this recombinant DNA [(for example, rHIV-1 (tat-, tk+) DNA] is then inserted into the modified T-cell line packaging system which was created in step 801. This produces in step 809 many copies of the recombinant virus [(for example, rHIV (tat-, tk+)]. Then in step 811, this recombinant

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virus is harvested and then injected into a human patient suffering from infection by wild-type HIV-1. Alternatively, a sample of the patient's blood may be taken and cultured with the recombinant virus (to infect T cells in the sample) and then the sample is injected back into the patient. Then, in step 813, a nucleoside analog such as Acyclovir is administered orally or Gancyclovir is injected. The standard therapeutic dosages of this nucleoside analog may typically be used several times over a period of time. While administering Acyclovir, blood samples from the patient may be taken to determine the progress of the treatment (by measuring the quantity of HIV present and/or the number of infected cells). As shown by the dashed lines in figure 8, further injections of the recombinant virus into the patient may be performed followed by further administrations of the nucleoside analog. Again, the progress of the treatment may be monitored by taking samples of the patient's blood and measuring the quantity of HIV present and/or the number of cells infected by HIV.

A particular implementation of the present invention will now be described by showing particular gene constructs which were used to create an embodiment of the recombinant human immunodeficiency virus of the present invention. Figure 2a shows a gene map of the proviral form of the HIV-1 genome. As is known, this genome is also similar to the HIV-2 genome and therefore the invention may be used to treat infections by HIV-2. As is known, the tat and rev regulatory genes of HIV-1 are mapped to two non contiguous regions of the genome, and the messenger RNA for tat is a spliced copy of each segment. The inactivation

strategy according to the present invention for inactivating tat seeks to disrupt the reading frame of the tat exon.

Figure 4 shows a particular plasmid, the plasmid pNL43 which contains two full copies of HIV from two different isolates of HIV (wild-type). These isolates are referred to as NY5 (5') and LAV (3') and were cloned directly from genomic DNA. See, generally, Adachi, A., et al., Journal of Virology, vol. 59, at pages 284-291 (1986). This plasmid pNL43 is used as the basis for the creation of the plasmid pNL43dBM, and the plasmid pNL43dBMtk.

Figure 5 shows a design strategy, according to one embodiment, for the HIV-1 (tat-) gene construct, which has been labelled pNL43dBM. The tat gene is inactivated, according to this strategy, by a 16 base pair deletion between BanII and MvaI restriction sites in the HIV-1 proviral DNA. This deletion renders the tat protein functionally inactive. As shown in Figure 5, the plasmid pNL43dBM (generally represented as plasmid 205 in Figure 3) is derived from the plasmid pNL43 (which is generally represented as plasmid 203 in Figure 3). A portion of the HIV-1 DNA in plasmid 203 about 70 nucleotides downstream from the SalI restriction site (in the tat gene) and 25 nucleotides downstream from the ATG codon was cut with BanII and MvaI restriction endonucleases. The fragments were then treated with S1 nuclease to digest the single-stranded portions of the fragments in order to blunt end them. The fragments were then bound to each other by the T4 DNA ligase enzyme. Deletion of the 16 base pair stretch was confirmed by sequencing. Also, see

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the paper by Shibata, et al. concerning research on tat mutants; Shibata, R., et al., Archives of Virology, V. 114, pages 243-250 (1990).

The tk gene from HSV-1 is obtained from the PvuII-Pvull fragment 207 shown in figure 7 which may be obtained from the plasmid 701 shown in figure 7. In particular in one embodiment, a portion of the HSV-1 genome, containing the tk gene (in the BamHII-BamHII fragment 703), was cloned into a plasmid vector, thus obtaining the plasmid 701. See generally the article by McKnight, S.L. and Grace, E.R., in Nucleic Acid Research, Vol. 8, starting at page 5981 (1980). The PvuII-PvuII fragment 207 shown in figure 7 is a fragment containing the complete tk gene that can be expressed under an HIV-1 promoter. This tk fragment 207 is inserted into the HIV proviral genome at the SalI site as shown in figure 6. Figure 6 illustrates a schematic representation of the construction of plasmids 209 and 210 into which the tk fragment is inserted. This insertion is produced by partial digestion by SalI followed by a fill-in reaction to produce blunt ends and the insertion and ligation of the PvuII-PvuII fragment 207. In particular, to make Plasmids 209 and 210, plasmids 203 and 205 respectively were cut with SalI (which is located just after the splice acceptor site for the tat/rev mRNA). The resulting single strand portions of DNA were filled-in with Klenow DNA polymerase producing blunt ends. The PvuII-PvuII fragment 207 of the HSV-1 subclone containing the tk gene (about 2 kilobase pairs in length) was inserted between the blunt ends of opened plasmids 203 and 205 by conventional blunt end ligation.

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Charts 1 and 2 illustrate various experimental results which demonstrate the in vitro effectiveness of the treatment method of the present invention and establish and verify the proper construction and functional operation of rHIV-1 (tat-, tk+) and the proper construction and operation of the modified T-cell lines. Chart 1 shows the results of assaying for the presence of p24 (an HIV-1 structural protein), assaying for RT (reverse transcriptase), observing cytopathic effect (CPE) and cell lysis in the presence of Acyclovir. In each case for the Jurkat tat cells (a modified cell-line of human T-cells with tat+), these cells were transfected with one of the three plasmids (pNL43, pNL43tk, or pNL43dBMtk as respectively indicated in rows 3, 4, & 5 of Chart 1) and then the cultures of the respective transfected cells were assayed for p24, RT, CPE and cell lysis (after treatment with 10 microMolar of Acyclovir). It would be expected that the modified T-cells transfected with pNL43BdMtk would produce rHIV-1 (tat-, tk+) and that these cells would experience cell death by lysis from treatments of Acyclovir (due to internal expression of the viral tk gene product). The results shown in chart 1 confirm this expectation as only 13% of the Jurkat (tat+) cells transfected with pNL43dBMtk were positive for HIV-1 p24 after only one treatment. 100% of the control Jurkat (tat+) cells untreated with Acyclovir but transfected with pNL43dBMtk were positive for HIV-1 (not shown in Chart 1). Accordingly some RT and CPE were also detected and observed. Moreover, the same (13%) number survived after cell lysis indicating that cell death by lysis did occur in the presence of Acyclovir. These are the same 13% cells that were

INSDOCID: <WO 9720052A1 I >

present in the IFA test. The potency of pNL43 as an HIV infective carrier is demonstrated by these assays. Control T cells lines (tat-) (i.e. not transformed to include the tat gent) transfected with tat-, tk- (e.g. pNL43dBM) or tat-, tk+ (e.g. pNL43dBMtk) did not synthesize virus-specific antigens and reverse transcriptase was not detected during an extended period of culturing of these control T cell lines. However, PCR (polymerase chain reaction) analysis did indicate the presence of HIV-1 specific sequences in these control cells, thereby verifying an effective transfection with the plasmids. As shown in Chart 1, the modified Jurkat (tat+) cells supported the production of infectious virus efficiently, including those cells transfected with pNL43, pNL43tk and pNL43dBMtk.

CHART 1

Test of pNL43 transfected Jurkat (tat+) cells for the replication of the progency virus and response to Acyclovir (10μM)

	Primary Transfectant Jurkat (tat+)			
Specimen Supernatant	IFA p24 (%+)	RT	CPE	Cell Lysis after Acyclovir
PBMC	-	-	-	-
Negative Jurkat	-	-	-	-
pNL43	45	++	-	No Effect
pNL43tk	43	+	- +	13
pNL43dBMtk	13	+	+	13
HIV (LAV)	47	++	-	No Effect

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Chart 2 demonstrates the infectiousness of the various forms of recombinant virus [including both of the control viruses produced by transfection of T-cells with pNL43 and pNL43tk and the desired recombinant virus rHIV-1 (tat-, tk+) produced by transfection of T-cells with pNL43dBMtk]. Note that these recombinant viruses were used for the infection of normal (not infected by HIV) fresh peripheral blood mononuclear cells (PBMC), which are known to be targets of wild-type HIV-1; these cells were also exposed to a wild-type HIV-1 (the HIV-1 LAV isolate) to verify their expected infectability. The PBMC exposed to rHIV-1 (tat-, tk+) were not killed by this virus while those PBMC cells exposed to the control recombinant viruses (shown in the columns pNL43 and pNL43tk) were killed as expected. In the case of the column labeled pNL43dBM, the supernatant from transfected T (tat+) cells (transfected with pNL43dBM) was applied to the culture containing the PBMC cells; it will be appreciated that it would be expected, based on the teachings of this invention, that no virus would be produced in this case, and no infection or cell lysis would occur. This expectation was also confirmed.

CHART 2

Infection of PBMC from 4 normal donors by the progency virus produced by the pNL43 series of virus produced from Jurkat tat+ cells.

INSDOCID: <WO 9720052A1 I >

Specimen number	pNL43	pNL43dBM	pNL43tk	pNL43dBMtk	HIV-1 (LAV)	Uninfected Control
1	CPE/cell lysis	no response	CPE/cell lysis	no response	CPE/cell lysis	Good
2	CPE/cell lysis	no response	CPE/cell lysis	no response	CPE/cell lysis	Good
3	CPE/cell lysis	no response	CPE/cell lysis	no response	CPE/cell lysis	Good
4	CPE/cell lysis	no response	CPE/cell lysis	no response	CPE/cell lysis	Good

For the columns labelled pNL43 and HIV-1 (LAV),CPE were observed in the infected cells within 3 to 5 days and all cells in these cultures died by 12-15 days. For the column labelled pNL43tk, CPE were observed by the 10th day in culture, although many of these cells remained healthy, and these cells were killed by applying 10 microMolar of Acyclovir.

This evidence demonstrates that the insertion of the tk gene is effective in producing cell death in the presence of a nucleoside analog such as Acyclovir or Gancyclovir and yet does not effect normal HIV specificity and infectivity. This also demonstrates that rHIV (tat-, tk+) is effective in infecting and killing T-cells specifically in the presence of the nucleoside analog. This also demonstrates that inactivation of the tat gene will prevent viral replication.

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In addition to the uses described herein for the recombinant virus (e.g. rHIV-1), this virus is useful for research and development into the molecular biology of HIV, and it can be used in animal studies for potentiating immune responses. This virus can also be used to test the response of an animal to the virus in a specially developed murine system which is transplanted with human lymphoid organs. These are mice with sever combined immunodeficiency (SCID-hu). Moreover, this virus can be used in regulated gene therapy strategies by adding a therapeutic gene to the rHIV, and injecting this modified rHIV into an animal and regulating over expression by injecting Acyclovir and injecting tat protein to cause expression of the therapeutic gene. The rHIV of the present invention may also be used in research and experimentation in the field of immunosuppression; for example, rHIV may be used to produce immunosuppresion to enhance organ transplantation or may be used to treat autoimmune disorders. In one case, rHIV may be introduced into an organ transplant patient and then the tat gene product may be injected intravenously while Acyclovir is introduced in the patient; in this manner, the infected CD4 lymphocytes may be selectively killed to suppress the immune system.

Various of the plasmids and cell lines described above have been deposited with the American Type Culture Collection (ATCC). In particular, samples of the modified Jurkat (tat+) T cell line have been deposited with the ATCC, 12301 Parklawn Drive, Rockville, MD, U.S.A. on October 3, 1995 under ATCC accession No. CRL 11987. Samples of the unmodified (control) Jurkat T cell (untransfected with plasmid

INSDOCID: <WO 9720052A1 I >

105) have been deposited with the ATCC, 12301 Parklawn Drive, Rockville, MD. USA on October 3, 1995 under ATCC Accession No. CRL 11988. Samples of the plasmids pNL43, in samples contained within Escherichia coli bacteria, have been deposited with the ATCC, 12301 Parklawn Drive, Rockville, MD, USA on October 3, 1995 under ATCC Accession No. 69928. Samples of the plasmid pNL43dBM, contained within bacteria Escherichia coli, have been deposited with the ATCC, 12301 Parklawn Drive, Rockville, MD, USA on October 3, 1995 under ATCC Accession No. 69927. Samples of the plasmid pNL43dBMtk, contained within bacteria Escherichia coli, have been deposited with the ATCC, 12301 Parklawn Drive, Rockville, MD, USA on October 3, 1995 under ATCC Accession No. 69929.

BNSDOCID: <WO___9720052A1_I_>

Applicant's or agent's file reference number: 02094.P001D	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 23, line 29. (Jurkat tat +)

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

ATCC
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
03 October 1995 (03.10.95)	ATCC NO. CRL 11987

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet [ ]

A Jurkat (tat +) T cell line (designated as 65AJT); a modified form of a human T cell line produced as described in the description at pages 12-14. This cell line is useful for packaging and/or producing a recombinant HIV as described in the description at pages 17-18.
Applicant hereby desires to avail itself of the benefit of the expert option under Rule 28(4) EPC.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

All designated states.

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer: H. Hill	Authorized officer

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INSDOCID: <WO 9720052A1 I >

Applicant's or agent's file reference number 02094.P001D	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 23, line 33. (Jurkat tat-)

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

ATCC
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
03 October 1995 (03.10.95)	ATCC NO. CRL 11988

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) — This information is continued on an additional sheet [ ]

A Jurkat T cell line (designated as 65BJ); a form of a human T cell line which is available and is useful for producing the Jurkat (tat+) T cell line as described in pages 12-14 of the description.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

All designated states.

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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BNSDOCID: <WO 9720052A1_I_>

Applicant's or agent's file reference number 02094.P001D	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 4 (pNL43)

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

ATCC
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
03 October 1995 (03.10.95)	ATCC NO. 69928

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet [ ]

Bacteria with pNL43 plasmid (designated as Escherichia coli strain DH5). These bacteria are produced as described in the description at pages 14 and 21. These bacteria are useful for producing modified versions of the pNL43 plasmid, such as pNL43dBM. Applicant hereby desires to avial itself of the benefit of the expert option under Rule 28(4) EPC.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

All designated states.

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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Authorized officer	Authorized officer

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INSDOCID: <WO 9720052A1 I >

Applicant's or agent's file reference number	02094.P001D	International application No

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 8 (pNL43dBM)

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

ATCC
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
03 October 1995 (03.10.95)	ATCC NO. 69927

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet [ ]

Bacteria with pNL43dBM plasmid (designated as Escherichia coli strain DH5). These bacteria are produced as described in the description at pages 14 and 21. These bacteria are useful for producing a modified version of the pNL43dBM plasmid, such as pNL43dBMtk.
Applicant hereby desires to avail itself of the benefit of the expert option under Rule 28(4) EPC.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

All designated states.

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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Applicant's or agent's file reference number	02094:P001D	International application No

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 12. (pNL43dBMtk)

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection (ATCC)

Address of depositary institution (*including postal code and country*)

ATCC
12301 Parklawn Drive
Rockville, Maryland 20582
United States of America

Date of deposit	Accession Number
03 October 1995 (03.10.95)	ATCC NO. 69929

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) — This information is continued on an additional sheet ☐

Bacteria with pNL43dBMtk plasmid (designated as Escherichia coli strain DH5). These bacteria are produced as described in the description at pages 14-15/21-22 These bacteria are useful for producing the plasmid pNL43dBMtk which may then be used to create a modified HIV ("rHIV").
Applicant hereby desires to avail itself of the benefit of the expert option under Rule 28(4) EPC.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

All designated states.

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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Authorized officer	Authorized officer

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INSDOCID: <WO __9720052A1 I >

These deposits with the ATCC provide for permanent availability of the progeny of these cell lines to the public on the issuance of the U.S. patent describing and identifying the deposit or the publication or laying open to the public of any US. or foreign patent applications, which ever comes first, and for availability of the progeny of these cell lines to one determined by the U.S Commission of patents and Trademarks to be entitled thereto according to 35 USC Section 122 and the Commissioner's rules pursuant thereto. The owner of the present application has agreed that if the cell lines on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable culture of the same cell line.

The present invention is not to be limited in scope by the microorganisms or cells deposited since the deposited embodiment is intended as a single illustration of one aspect of the invention and any microorganisms or cells or modified viruses which are functionally equivalent are within the scope of this invention. Indeed various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

Although the present invention has been described with preferred embodiments, it is to be understood that modifications and variations may be resorted to, without departing from the spirit and scope of this

BNSDOCID: <WO 9720052A1 | >

invention, as those skilled in the art will readily understand.

Various forms of the HIV virus may be modified, as described herein, to include the tk gene and to incapacitate the tat gene to produce an rHIV virus of the present invention. A search of the GenBank database may be performed to obtain these HIV viruses. As is well known, the GenBank includes many nucleotide sequences and is maintained by the National Center for Biotechnology Information (National Library of Medicine, National Institutes of Health). The GenBank is available through the home page of the National Center for Biotechnology Information on the Works Wide Web (at the Uniform Resource Locator http://www.ncbi.nlm.nih.gov/).

A search of the GenBank for HIV which contain proviral sequences similar to a portion of the pNL43 sequence was performed to obtain examples of forms of HIV which are expected to be compatible with the methods of the present invention such that these forms of HIV may be modified to produce a corresponding form of rHIV. Listed below in the accompanying Sequence Listing are portions of 15 examples of these forms of HIV. The following chart (CHART 3) identifies these particular examples by referring to the GenBank's accession number. The portion of the pNL43 sequence used to search the GenBank is shown in the accompanying Sequence Listing as "M19921" (SEQ.ID No: 1).

INSDOCID: <WO__9720052A1_I_>

CHART 3

Accession No.	Other Description	SEQ. ID NO.
M19921	HIVNL43	1
L31963	HIVTH475A	2
K03455	HIVHXB2CG	3
U12055	HIV1U12055	4
K02013	HIVBRUCG	5
M15654	HIVBH102	6
I04549	European Patent 0187041	7
X01762	REHTLV3	8
I07983	European patent 0185444	9
K02083	HIVPV22	10
M17449	HIVMNCG	11
I12142	U.S.Patent 5,420,030	12
M38429	HIVJRCSF	13
L02317	HIV1SG3X	14
M96155	HIV1PROV	15

BNSDOCID: <WO__9720052A1_I_>

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANTS: Syed Zaki Salhuddin and Nickolas Chelyapov

(ii) TITLE OF INVENTION: Recombinant HIV and Modified Packaging Cells and Method for Testing Acquired Immune Deficiency Syndrome

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:

(A) ADRESSEE: Blakely, Sokoloff, Taylor & Zafman

(B) STREET: 12400 Wilshire Boulevard, Suite 700

(C) CITY: Los Angeles

(D) STATE: California

(E) COUNTRY: U.S.A.

(F) ZIP: 90025

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 inch, double-sided, double-density disk.

(B) OPERATING SYSTEM: Apple MacIntosh.

INSDOCID: <WO__9720052A1_I_>

(C) SOFTWARE: Microsoft Word Version 5.1a.

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: James Scheller, Jr.

(B) REGISTRATION NUMBER: 31,195

(C) REFERENCE/DOCKET NUMBER: 02094.P001

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (408) 720-8598.

(B) TELEFAX: (408) 720-9397.

BNSDOCID: <WO 9720052A1 I >

(2) INFORMATION FOR SEQ. ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: M19921; Human immunodeficiency virus type 1, NY5/BRU (LAV-1) recombinant clone pNL4-3.

(xi) Sequence Description: SEQ.ID NO: 1:

GGGATACTTG GCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATGACA 240

INSDOCID: <WO__ 9720052A1_I_>

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG 300

AACAGTCAGA CTCATCAAGC TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: L31963; Human Immunodeficiency virus type 1 (individual isolate: TH4-7-5) gene.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 2:

GGGATACTTG GCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCAACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA CTTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATGACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG 300

AACAGTCAGA CTCATCAAGC TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

INSDOCID: <WO 9720052A1 I >

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: K03455; Human immunodeficiency virus type 1 (HXB2), complete genome; HIV1/HTLV-III/LAV reference genome.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 3:

GGGATACTTG GCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCATCAG 300

AACAGTCAGA CTCATCAAGC TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: U12055; Human immunodeficiency virus type 1 isolate LW12.3 from infected lab worker, complete genome.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 4:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTACCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCT TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

INSDOCID: <WO 9720052A1 I >

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

AGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: K02013; Human immunodeficiency virus type 1, isolate BRU, complete genome (LAV-1).

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 5:

BNSDOCID: <WO 9720052A1 >

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCAACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA CTTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCACAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

INSDOCID: <WO 9720052A1 I >

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: M15654; Human immunodeficiency virus type 1, isolate BH10, genome.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 6:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: I04549; Sequence 11 from patent EP 0187041

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 7:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

INSDOCID: <WO___9720052A1_I_>

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: X01762; Human T-cell leukemia type III (HTLV-III) proviral genome (AIDS virus for acquired immune deficiency syndrome)

RNSDOCID: <WO_ 9720052A1_I_>

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO. 8:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

INSDOCID: <WO___9720052A1_I_>

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: I079083; Sequence 1 from patent EP 0185444

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 9:

GGGATACTTG GCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: K02083; Human immunodeficiency virus type 1, isolate PV22, complete genome (H9/HTLV-III proviral DNA).

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 10:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT GCAACAACTG CTGTTTATCC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCGTTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

INSDOCID: <WO__9720052A1_I_>

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GGCAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: M17449; HIV-1, isolate MN

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 11:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT ACAACAACTG CTGTTTATTC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCATTAT TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAGACT 180

GCTTGTACCA CTTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCACAAAA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCCTGAA 300

GACAGTCAGA CTCATCAAGT TTCTCTACCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

INSDOCID: <WO__9720052A1_I_>

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: I12142; Sequence 1 from U.S. Patent No: 5,420,030.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 12:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATTCT ACAACAACTG CTGTTTATTC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCATTAT TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAGACT 180

GCTTGTACCA CTTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCACAAAA 240

AAAGCCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCCTGAA 300

GACAGTCAGA CTCATCAAGT TTCTCTACCA AAGCAGTAAG TAGTACATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: M38429; Human immunodeficiency virus type 1, isolate JRCSF, complete genome.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 13:

GGGATACTTG GGCAGGAGTG GAAGCCATAA TAAGAATACT GCAACAGCTG CTGTTTATTC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCATTAC TCGACAGAGG AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG CCTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAGACT 180

INSDOCID: <WO___9720052A1_I_>

GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCCTTCATT GCCAAGTTTG TTTCACAACA 240

AAAGGCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GACAGTCAGA CTCATCAAGT TTCTCTACCA AAGCAGTAAG TAGTGCATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: L02317; Human immunodeficiency virus type 1

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 14:

GGGATACTTG GGCAGGAGTG GAAGCCCTAG TAAGAACTCT GCAACAACTG CTGTTTACTC 60

TTTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCATTAC TCAACGAAGA AGAGCAAGAA 120

ATGGAGCCAG TAGATCCTAG ACTAGAGCCC TGGAAGCATC CAGGAAGCCA GCCTAAAACT 180

GCTTGTACCA AATGCTATTG TAAAAAGTGT TGCTTACATT GCCAAGTTTG TTTCATGACA 240

AAAGGCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG AGCTCCTCAA 300

GACAGTCAGA CTCATCAAGC TTCTCTATCA AAGCAGTAAG TAGTGCATGT A 351

(2) INFORMATION FOR SEQ. ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double-stranded

INSDOCID: <WO___9720052A1_I_>

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:

(A) DESCRIPTION: GenBank Accession No: M96155; Human immunodeficiency virus type 1 proviral DNA encoding the vif, vpr, vpu, env, tat, rev, and nef genes, complete cds, and 3' LTR.

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO: 15:

GGGATACTTG GACAGGAGTG GAAGCCTTAA TAAGAATTCT GCAACAACTG CTGTTTATTC 60

ATTTCAGAAT TGGGTGTCGA CATAGCAGAA TAGGCATTAT TCAACACAGG AGAACAAGAA 120

ATGGAGCCAG TAAATCCTAG CCTAGAGCCC TGGAAGCATC CAGGAAGTCA GCCTAAAACT 180

GCTTGTACCA ATTGCTATTG CAAAAAATGT TGCTTTCATT GCCAAGCTTG TTTCATAACA 240

AAAGGCTTAG GCATCTCCTA TGGCAGGAAG AAGCGGAGAC AGCGACGAAG ACCTCCTCAA 300

GACAGTCAGA CTCATCAAGT TTCTCTATCA AAGCAGTAAG TAGTACATGT A 351

BNSDOCID: <WO___9720052A1_I_>

CLAIMS

1. A method for treating acquired immune deficiency disease in a human caused by human immunodeficiency virus (HIV), said method comprising:

administering a composition comprising a modified human immunodeficiency virus (modified HIV) having a gene which encodes a viral thymidine kinase, said modified HIV being unable to express at least one functional regulatory gene product; and

administering a nucleoside analog.

2. A method as in claim 1 wherein said nucleoside analog comprises one of Acyclovir or Gancyclovir.

3. A method as in claim 1 wherein said functional regulatory gene product is the expressed gene product of the tat gene of the HIV genome.

4. A method as in claim 1 wherein said functional regulatory gene product is the expressed gene product of the rev gene of the HIV genome.

5. A method as in claim 1 wherein said nucleoside analog is selected from the group comprising Acyclovir and Gancyclovir, and wherein said modified HIV is unable to express functional gene products of the tat and rev genes of the HIV genome.

6. A method as in claim 1 wherein said modified HIV is unable to replicate in a cell without the presence of a wild-type HIV.

INSDOCID: <WO_ 9720052A1_I >

7. A method as in claim 1 wherein said modified HIV is harvested from a modified mammalian cell having a tat HIV regulatory gene in the genome of said modified mammalian cell.

8. A method as in claim 7 wherein said modified mammalian cell is a human T-cell.

9. A method as in claim 7 wherein said modified mammalian cell is a human B-cell.

10. A method as in claim 1 wherein said modified HIV infects human T-cells which have been previously infected by wild-type HIV.

11. A method of treating human immumodeficiency virus (HIV) infection in a human, said method comprising:

administering a composition comprising a modified human immunodeficiency virus (modified HIV) having a gene, which when expressed in a cell which is infected by HIV, causes at least a portion of cells which are infected by HIV to be killed.

12. A method as in claim 11 further comprising:

administering an agent which in combination with an expressed product of said gene causes said portion of cells to be killed.

13. A method as in claim 12 wherein said agent is a nucleoside analog and wherein said gene encodes a kinase enzyme.

14. A method as in claim 11 wherein said modified HIV is unable to replicate in a cell without the presence of a wild-type HIV.

15. A method as in claim 11 wherein said modified HIV is unable to express at least one functional regulatory gene product.

16. A method as in claim 15 wherein said functional regulatory gene product comprises one of the tat and the rev genes of the HIV genome.

17. A method as in claim 16 further comprising:

harvesting said modified HIV from a modified mammalian cell-line having said one of the tat and the rev genes in the genome of said modified mammalian cell-line.

18. A modified human immunodeficiency virus (modified HIV) comprising in its genome a chimeric recombinant gene construction including a foreign gene, said chimeric recombinant gene construction being effective in human cells infected with a human immunodeficiency virus (HIV) to express a gene product coded by the foreign gene, said gene product being capable of causing at least a portion of human cells infected by HIV to be killed.

19. A modified HIV as in claim 18 wherein said modified HIV is unable to express at least one functional regulatory gene product of the genome of HIV.

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20. A modified HIV as in claim 19 wherein said at least one functional regulatory gene product is selected from the group consisting of the tat and the rev genes in the genome of HIV.

21. A modified HIV as in claim 18 wherein said gene product is a kinase enzyme.

22. A modified HIV as in claim 21 wherein said kinase enzyme is thymidine kinase.

23. A modified HIV as in claim 21 wherein the genome of said modified HIV does not include a functional regulatory gene which codes for a functional regulatory gene product of the genome of HIV.

24. A modified HIV as in claim 23 wherein said functional regulatory gene is selected from the group consisting of the tat and the rev genes in the genome of HIV.

25. A modified HIV as in claim 20 wherein said gene product is a kinase enzyme and wherein said modified HIV is unable to replicate in a cell without the presence of a wild-type HIV.

26. A modified HIV as in claim 20 wherein said chimeric recombinant gene construction includes promoter and control sequences.

27. A modified HIV as in claim 20 wherein said modified HIV is harvested from a modified mammalian cell having at least one of the tat and the rev genes in the genome of said modified mammalian cell.

BNSDOCID: <WO__9720052A1_I_>

28. A modified HIV as in claim 27 wherein said gene product is thymidine kinase enzyme and wherein said gene product kills said portion of human cells wherein combined with a nucleoside analog.

29. A method for treating acquired immune deficiency disease in a human caused by human immunodeficiency virus (HIV), said method comprising:

administering to said human a composition comprising one of Acyclovir or Gancyclovir; and

administering a composition capable of providing viral thymidine kinase in said human.

30. A method as in claim 29 wherein said composition capable of providing viral thymidine kinase comprises a modified human immunodeficiency virus (modified HIV) having a gene which encodes said viral thymidine kinase, said modified HIV being unable to express at least one functional regulatory gene product.

31. A method as in claim 29 wherein said composition capable of providing viral thymidine kinase comprises a saline solution of viral thymidine kinase, and wherein said step of administering comprises injecting said solution into a patient.

32. A method as in claim 30 wherein said at least one functional regulatory gene product is the expressed gene product of the tat gene of the HIV genome.

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33. A method as in claim 30 wherein said modified HIV is unable to replicate in a cell without the presence of a wild-type HIV.

34. A method for producing a modified human immunodeficiency virus (modified HIV) comprising:

producing a plurality of modified HIV in a modified mammalian cell, said modified HIV having a gene which encodes a foreign gene product and being unable to express at least one functional regulatory gene product, said modified mammalian cell having a gene which encodes said at least one functional regulatory gene product.

35. A method as in claim 34 further comprising harvesting said plurality of modified HIV from said modified mammalian cell.

36. A method as in claim 35 wherein said step of producing is performed in tissue culture and wherein said modified mammalian cell is from a human T cell-line.

37. A method as in claim 36 wherein said foreign gene product is a kinase enzyme and said functional regulatory gene product is a tat protein of a human immunodeficiency virus.

38. A method as in claim 37 wherein said kinase enzyme is thymidine kinase.

39. A modified human immunodeficency virus (modified HIV) produced by a process comprising:

culturing a modified mammalian cell, said modified mammalian cell having a gene which encodes at least one functional regulatory gene product of HIV;

producing a plurality of modified HIV in said modified mammalian cell, said modified HIV having a gene which encodes a viral thymidine kinase and being unable to express said at least one functional regulatory gene product of HIV.

40. A modified HIV produced by a process as in claim 39, wherein prior to culturing said modified mammalian cell, said modified mammalian cell was transfected with a vector containing the genome of said modified HIV.

41. A modified HIV produced by a process as in claim 39 further comprising harvesting said plurality of modified HIV from said modified mammalian cell.

42. A modified HIV produced by a process as in claim 39 wherein said modified mammalian cell is from a human T cell line.

43. A modified HIV produced by a process as in claim 39 wherein said at least one functional regulatory gene product of HIV is the tat gene product.

44. A modified HIV produced by a process as in claim 39 wherein said modified HIV is unable to replicate in a mammalian cell without the presence of wild-type HIV.

INSDOCID: <WO___9720052A1_I_>

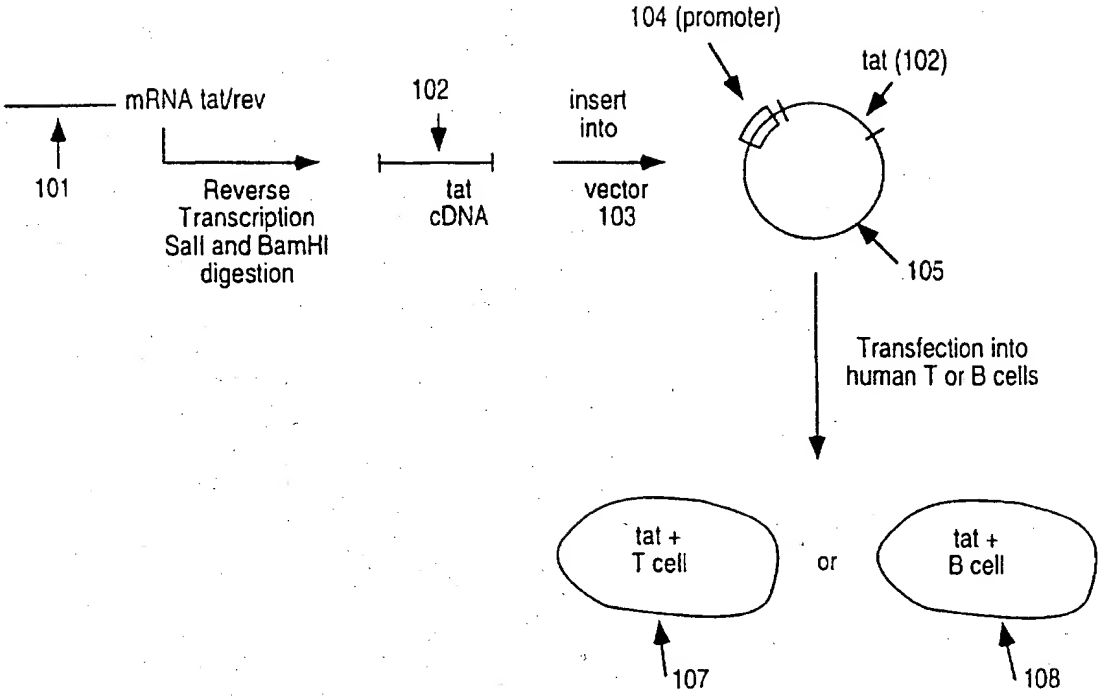

104 (promoter)
tat (102)
mRNA tat/rev
102
insert
into
101
Reverse
Transcription
SalI and BamHI
digestion
tat
cDNA
vector
103
105
Transfection into
human T or B cells
tat +
T cell
or
tat +
B cell
107
108

FIG. 1

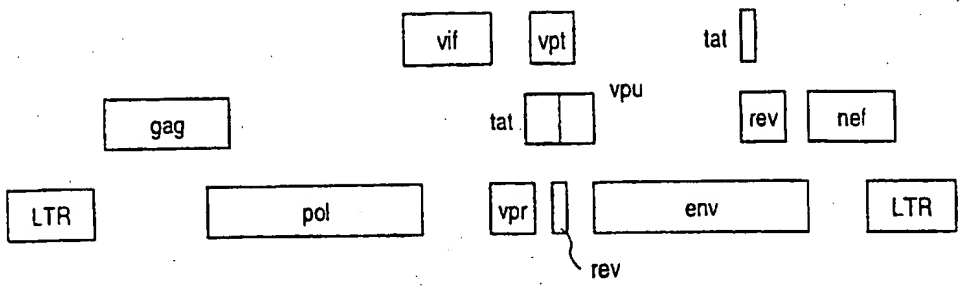


FIG. 2A

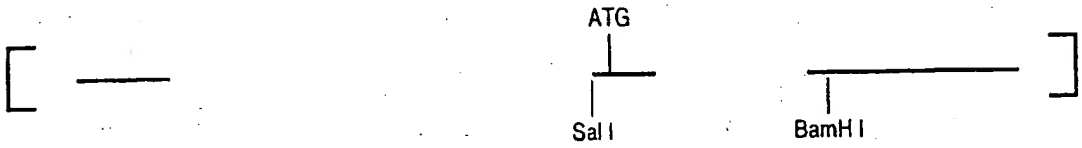


FIG. 2B

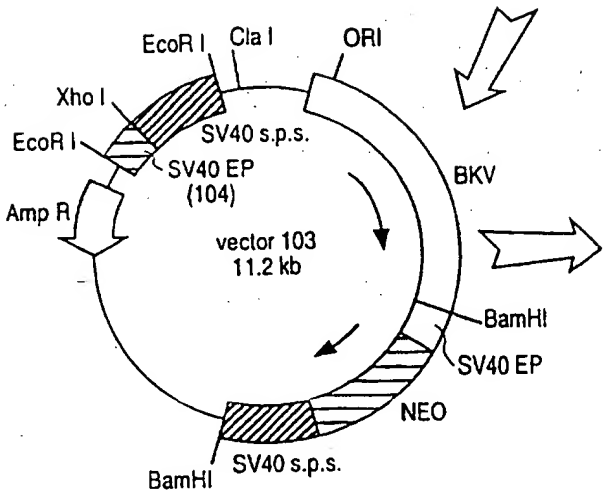


FIG. 2C

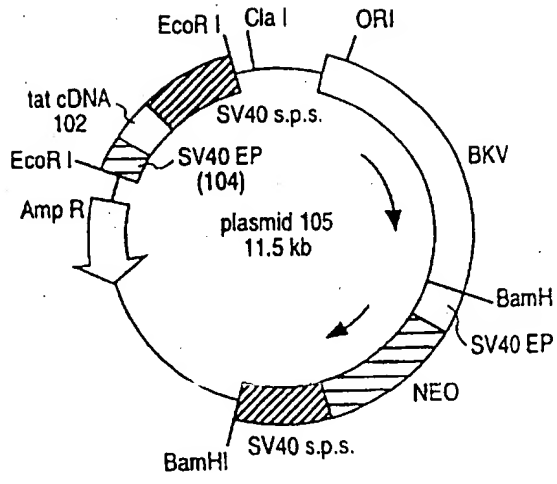


FIG. 2D

INSDOCID: <WO__9720052A1_I_>

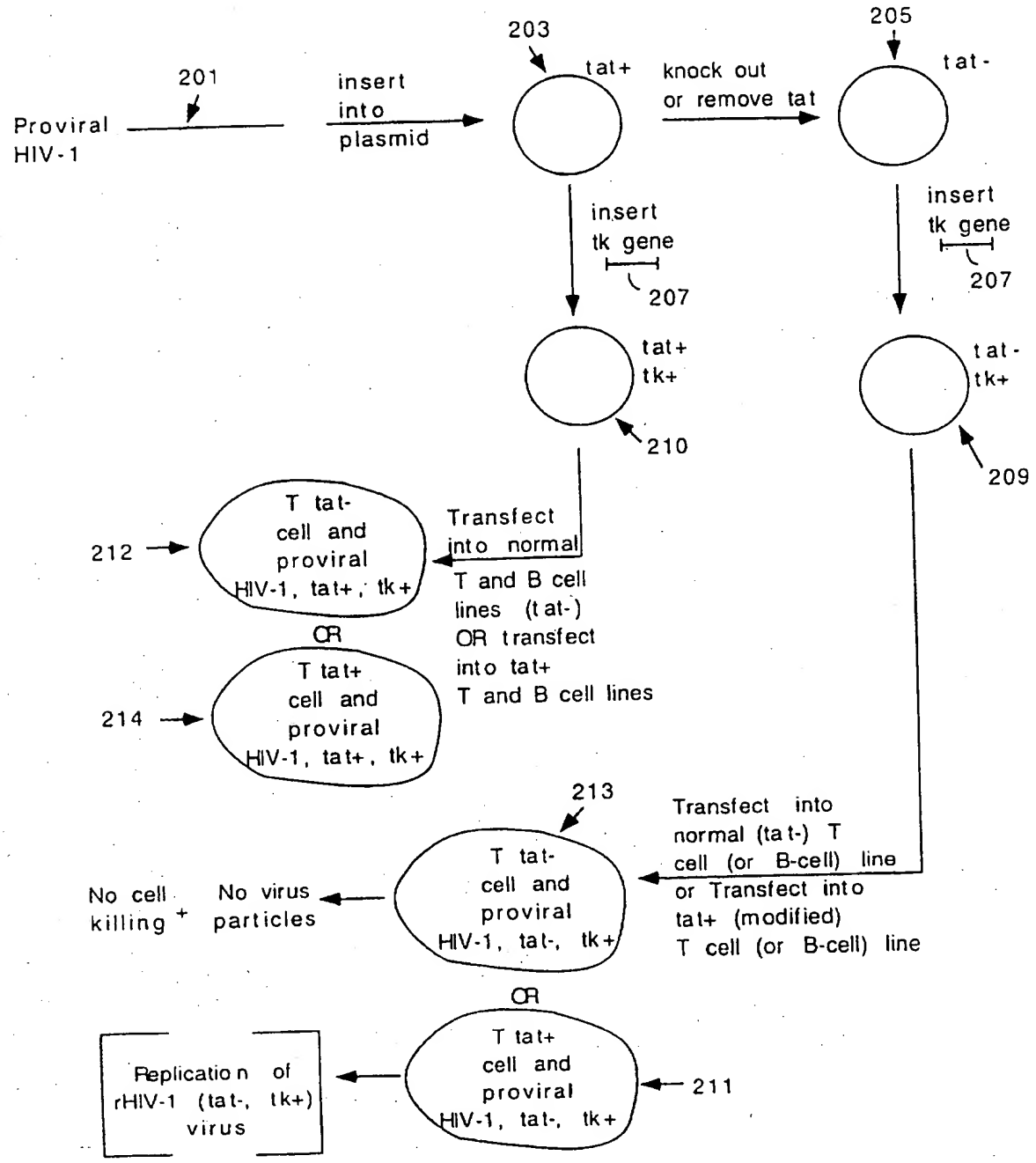
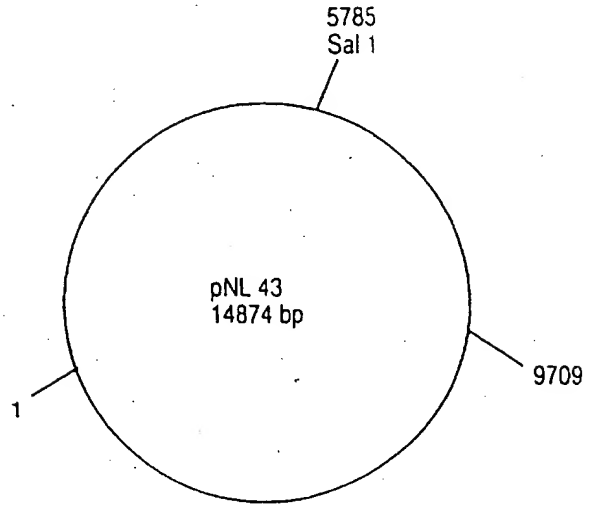


FIG. 3

BNSDOCID: <WO__9720052A1_I_>

HIV-1 CONTAINING PLASMID pNL 43



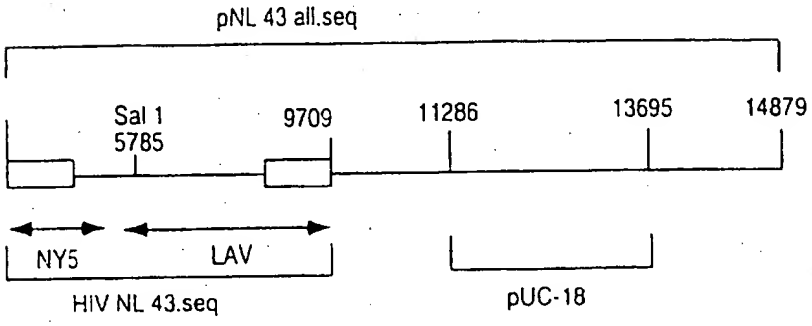


FIG. 4

INSDOCID: <WO 9720052A1 I >

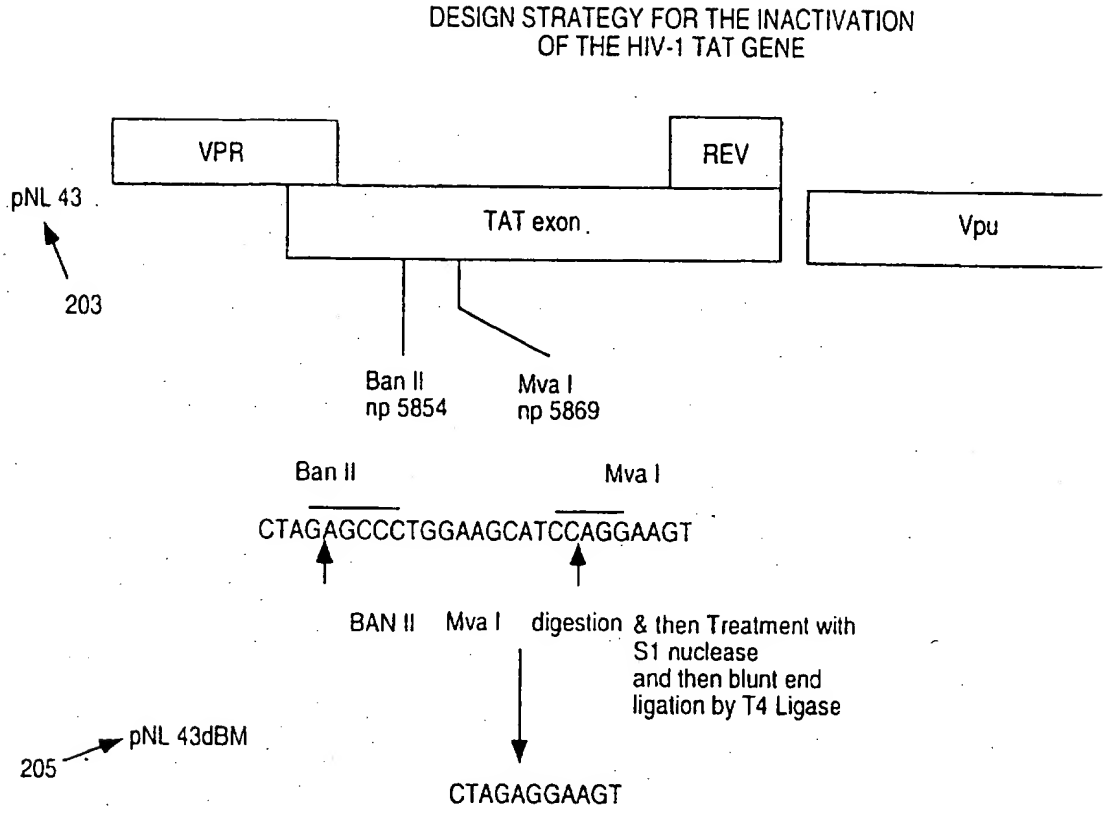

DESIGN STRATEGY FOR THE INACTIVATION
OF THE HIV-1 TAT GENE
VPR
REV
pNL 43
TAT exon
Vpu
203
Ban II
np 5854
Mva I
np 5869
Ban II
Mva I
CTAGAGCCCTGGAAGCATCCAGGAAGT
BAN II
Mva I
digestion
& then Treatment with
S1 nuclease
and then blunt end
ligation by T4 Ligase
pNL 43dBM
205
CTAGAGGAAGT

FIG. 5

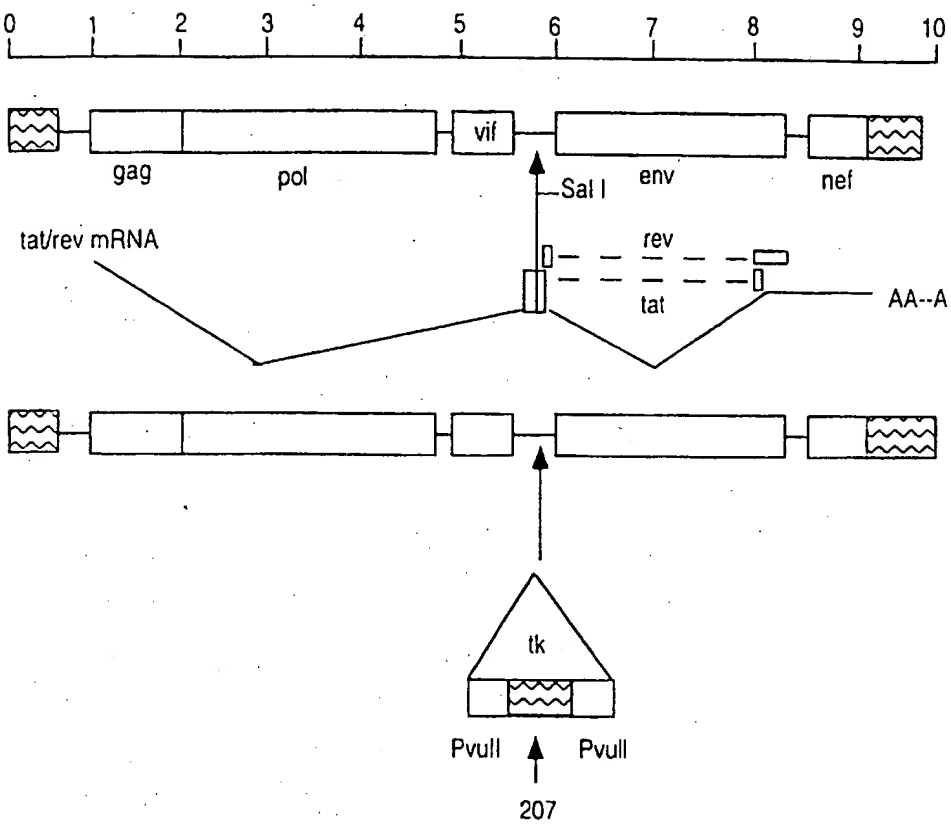

0
1
2
3
4
5
6
7
8
9
10
vif
gag
pol
Sal I
env
nef
tat/rev mRNA
rev
tat
AA--A
tk
PvuII
PvuII
207

FIG. 6

PLASMID pHSV-106 CONTAINING THE HSV-1 TK GENE

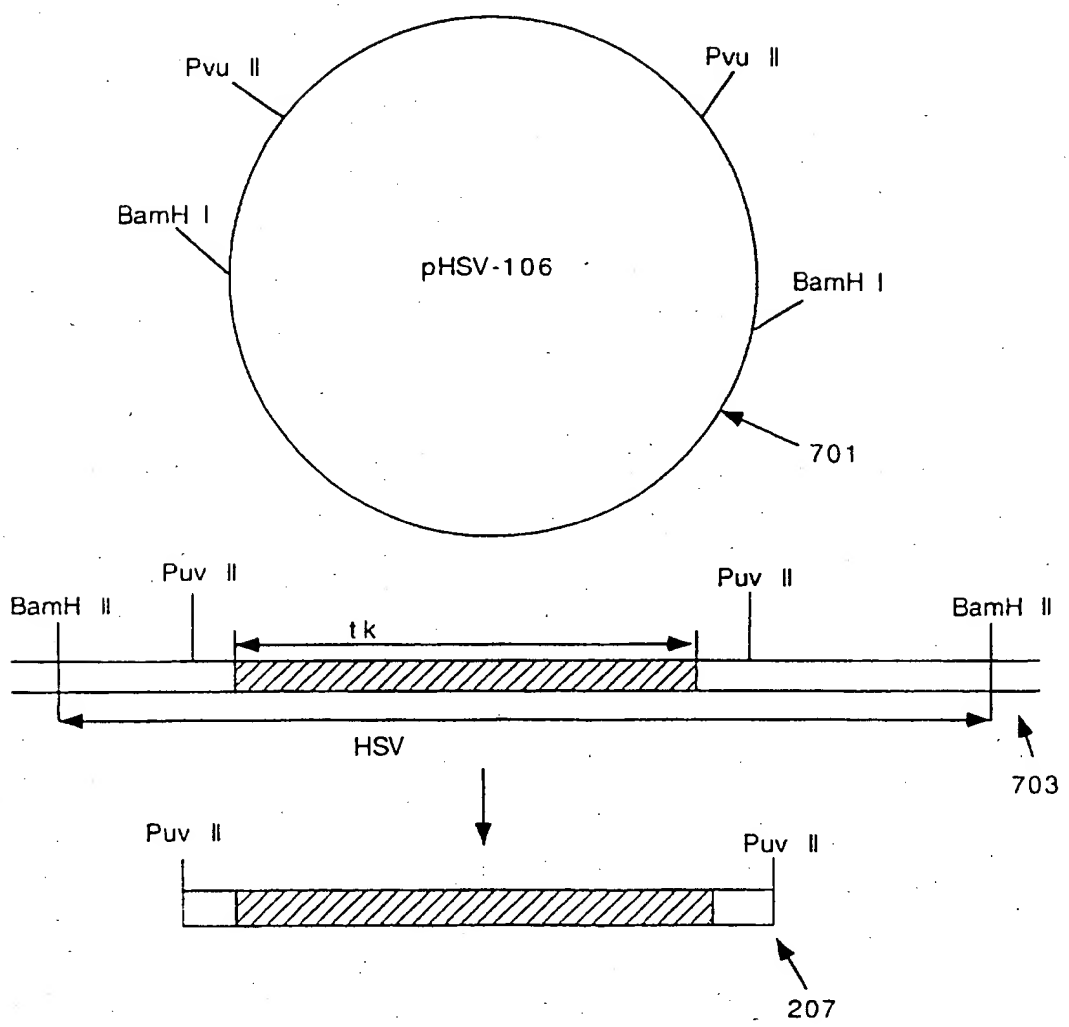


FIG. 7

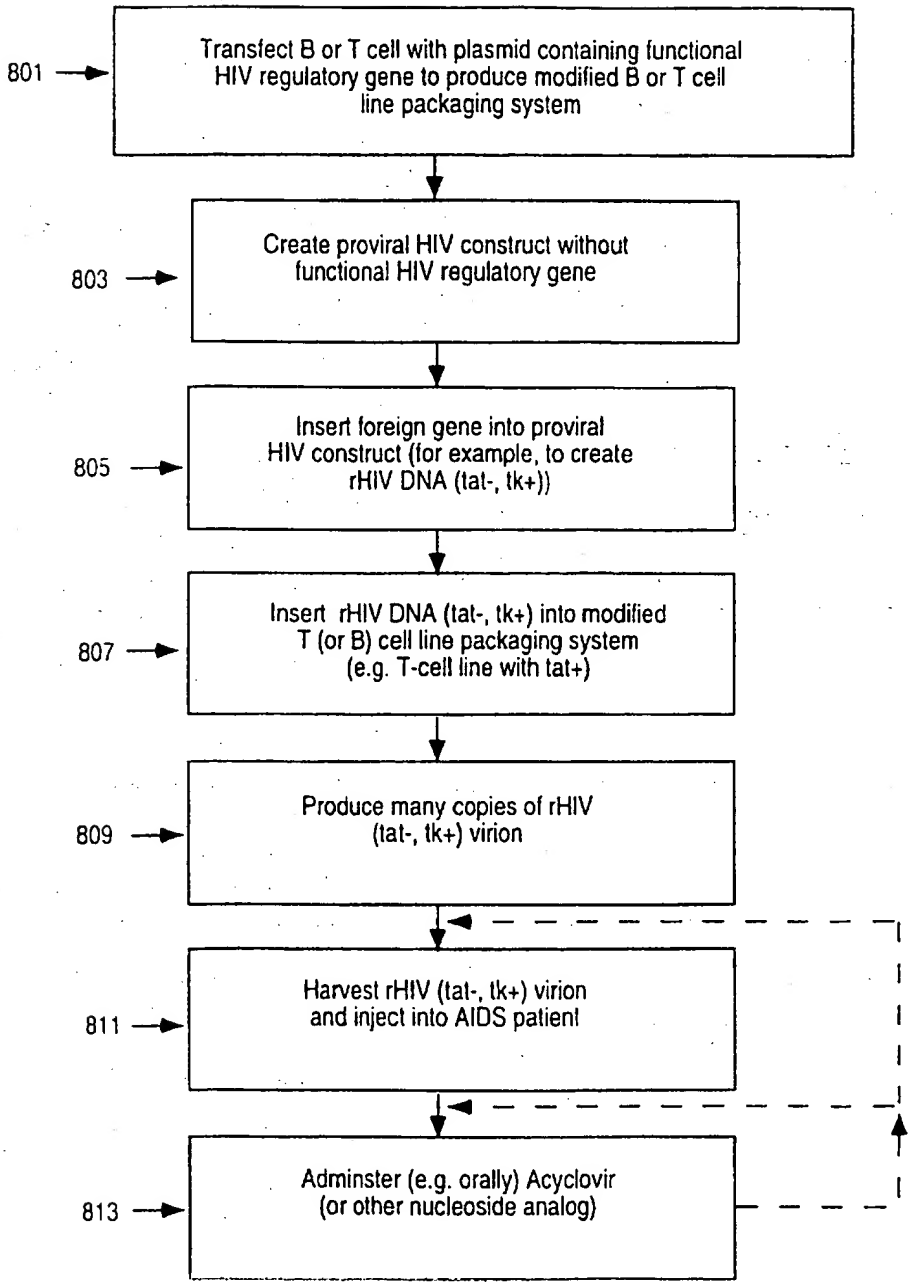


FIG. 8

INSDOCID: <WO___9720052A1_I_>

ACYCLOVIR SODIUM (Parenteral) {Zovirax}

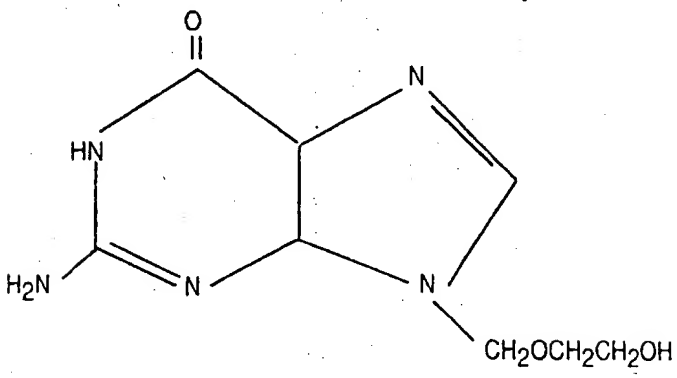


FIG. 9

BNSDOCID: <WO___9720052A1_I_>

INTERNATIONAL SEARCH REPORT

Internationa plication No
PCT/US 96/18885

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/48 C12N15/86 A61K48/00 A61K35/76

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 306 631 A (HARRISON GAIL ET AL) 26 April 1994 see the whole document ---	
A	US 5 428 143 A (BERGER EDWARD A ET AL) 27 June 1995 see the whole document ---	
A	HUMAN GENE THERAPY, vol. 2, no. 1, 1991, pages 53-60, XP000651800 HARRISON G S ET AL: "Activation of a diphteria toxin A gene by ..." see the whole document -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 1997	07. 05. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Halle, F

Form PCT/ISA/210 (second sheet) (July 1992)

INSDOCID: <WO___9720052A1_I_>

INTERNATIONAL SEARCH REPORT

Inte ional application No.
PCT/US 96/ 18885

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-17,29-38
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although these claims are directed to a method of treatment of the human or animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

BNSDOCID: <WO___9720052A1_I_>

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internation. pplication No
PCT/US 96/18885

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5306631 A	26-04-94	US 5554528 A	10-09-96
		US 5585254 A	17-12-96
US 5428143 A	27-06-95	US 5206353 A	27-04-93
		AU 4069089 A	19-02-90
		EP 0428603 A	29-05-91
		IL 91070 A	30-03-95
		WO 9001035 A	08-02-90
		US 5587455 A	24-12-96

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7. The conditionally replicating viral vector of claim 4, wherein the catalytic domain of said ribozyme cleaves the nucleotide sequence of SEQ ID NO: 3.

8. The conditionally replicating viral vector of claim 4, wherein said ribozyme is encoded by a sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5.

9. The conditionally replicating viral vector of claim 5, wherein said wild-type strain of virus comprises a nucleotide sequence encoded by SEQ ID NO: 1 and said vector, if DNA, comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18, and said vector, if RNA, comprises a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18.

10. The conditionally replicating viral vector of claim 5, wherein said vector lacks the *tat* gene and its splice site from the genome of the human immunodeficiency virus, wherein said human immunodeficiency virus is wild-type.

11. The conditionally replicating viral vector of claim 10, wherein, in place of said *tat* gene and its splice site, said vector comprises a triple anti-Tat ribozyme cassette, wherein the catalytic domain of each ribozyme of the triple ribozyme cassette cleaves a different site on a wild-type human immunodeficiency viral nucleic acid molecule.

12. The conditionally replicating viral vector of claim 11, wherein said wild-type human immunodeficiency viral nucleic acid molecule comprises *tat* and the

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catalytic domain of each ribozyme of the triple ribozyme cassette cleaves a different site within *tat*.

13. The conditionally replicating viral vector of claim 11, wherein the catalytic domain of each ribozyme cleaves a nucleotide sequence in a region of a nucleic acid molecule of wild-type human immunodeficiency virus for which there is no ribozyme-resistant counterpart in the vector, itself.

14. A conditionally replicating viral vector, which is characterized by a capacity to replicate only in a host cell that is permissive for replication of said vector, and wherein said vector comprises at least one nucleic acid sequence, the presence, transcription or translation of which confers to a host cell, which is infected with said vector, a selective advantage over a cell infected with a wild-type strain of virus corresponding to the virus from which said vector was derived or a helper.

15. The conditionally replicating viral vector of claim 14, wherein said at least one nucleic acid sequence comprises a nucleotide sequence encoding a multidrug resistance.

16. The conditionally replicating viral vector of claim 15, wherein said vector is derived from a human immunodeficiency virus.

17. The conditionally replicating viral vector of claim 15, wherein said vector is derived from a Togaviridae.

18. The conditionally replicating viral vector of claim 16, wherein said at least one nucleic acid sequence comprises a nucleotide sequence selected from the group

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consisting of a nucleotide sequence encoding a mutant protease and a nucleotide sequence encoding a mutant reverse transcriptase.

19. The conditionally replicating viral vector of claim 1, wherein said vector further comprises at least one additional nucleic acid sequence, the presence, transcription or translation of which confers to a host cell, which is infected with said vector, a selective advantage over a cell infected with a wild-type strain of virus corresponding to the virus from which said vector was derived or a helper.

20. The conditionally replicating viral vector of claim 19, wherein said at least one additional nucleic acid sequence comprises a sequence encoding a multidrug resistance.

21. The conditionally replicating viral vector of claim 19, wherein said vector is derived from a human immunodeficiency virus.

22. The conditionally replicating viral vector of claim 19, wherein said vector is derived from a Togaviridae.

23. The conditionally replicating viral vector of claim 21, wherein said at least one additional nucleic acid sequence comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence encoding a mutant protease and a nucleotide sequence encoding a mutant reverse transcriptase.

24. The conditionally replicating viral vector of claim 23, wherein said vector is selected from the group consisting of crHIV-1.1, crHIV-1.11, crHIV-1.12, and crHIV-1.111.

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25. A pharmaceutical composition comprising a vector of claim 1 and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a vector of claim 14 and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising a vector of claim 19 and a pharmaceutically acceptable carrier.

28. A host cell comprising a vector of claim 1.

29. A host cell comprising a vector of claim 14.

30. A host cell comprising a vector of claim 19.

31. A vector, wherein said vector, if DNA, comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18 and wherein said vector, if RNA, comprises a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18.

32. A method of engendering a vector, which is derived from a wild-type human immunodeficiency virus and which is capable of replicating only in a host cell that is permissive for replication of said vector, with a ribozyme, which is comprised within or encoded by said vector and which cleaves a nucleic acid of a wild-type human immunodeficiency virus but not the vector, itself, and its transcripts, if any, which method comprises:

(a) obtaining a vector, which is derived from a wild-type human immunodeficiency virus and which is

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capable of replicating only in a host cell that is permissive for replication of said vector; and

(b) incorporating into the vector of (a) a nucleic acid sequence, which comprises or encodes, in which case it also expresses, a ribozyme, the catalytic domain of which cleaves a nucleic acid of a wild-type human immunodeficiency virus but not the vector, itself, and its transcripts, if any.

33. The method of claim 32, wherein step (b) comprises:

(i) deleting from said vector a nucleotide sequence comprising or encoding the U5 sequence of the wild-type human immunodeficiency virus; and

(ii) inserting into the vector of (i) a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18, if the vector is DNA, and a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18, if the vector is RNA.

34. The method of claim 32, wherein said vector replicates in a host cell permissive for replication of said vector more than once.

35. A method of modifying a vector, which method comprises:

(a) obtaining a vector; and

(b) introducing into the vector of (a) a nucleotide sequence selected from the group consisting of the DNA sequences of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18, if the vector is DNA, and a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 4, 5, 6, 7, 15, 16, 17 and 18, if the vector is RNA.

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36. A method of propagating and selectively packaging a conditionally replicating vector without using a packaging cell line, which method comprises:

(a) contacting the conditionally replicating vector with a cell capable of being infected by another vector, which is the same type of vector as the conditionally replicating vector and which differs from the conditionally replicating vector by being wild-type for replication competency;

(b) contacting the cell of (a) with said another vector of (a); and

(c) culturing the cell of (b) under conditions conducive to the propagation of said conditionally replicating vector.

37. An isolated and purified nucleic acid molecule selected from the group consisting of a DNA molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18 and a RNA molecule comprising a nucleotide sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 6, 7, 15, 16, 17 and 18.

38. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 1, the presence, transcription or translation of which inhibits the replication of the wild-type strain of virus in the host cell.

39. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 14, the presence, transcription or

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translation of which inhibits the replication of the wild-type strain of virus in the host cell.

40. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 19, the presence, transcription or translation of which inhibits the replication of the wild-type strain of virus in the host cell.

41. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 13, the presence, transcription or translation of which inhibits the replication of the wild-type strain of virus in the host cell.

42. The method of claim 41, which additionally comprises contacting the host cell with an agent selected from the group consisting of a cytotoxic drug, a protease inhibitor, and a reverse transcriptase inhibitor.

43. A method of inhibiting the replication of a wild-type strain of a virus in a host cell, which method comprises contacting the host cell, which is capable of being infected with the wild-type strain of the virus, with a vector of claim 18, the presence, transcription or translation of which inhibits the replication of the wild-type strain of virus in the host cell.

44. The method of claim 43, which additionally comprises contacting the host cell with an agent selected from the group consisting of a cytotoxic drug, a protease inhibitor, and a reverse transcriptase inhibitor.

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45. The method of claim 38, wherein said virus causes cancer.

46. The method of claim 38, wherein said host cell has not yet come into contact with said wild-type strain of virus and wherein said method additionally comprises contacting said host cell with a helper-expression vector, wherein said helper-expression vector complements said conditionally replicating viral vector for its inability to replicate.

47. The method of claim 46, wherein said helper-expression vector complements said conditionally replicating viral vector in a cell-specific manner.

48. A method of expressing a gene of interest in a host cell, which method comprises:

(a) contacting said host cell with a conditionally replicating viral vector of claim 1, wherein said viral vector further comprises the gene of interest and is capable of expressing the gene in said host cell, and a helper; and (b) expressing in said host cell the gene of interest.

49. The method of claim 48, wherein said expression of said gene of interest in said host cell inhibits replication of a wild-type virus in said host cell.

50. A method of expressing a gene of interest in a host cell, which method comprises:

(a) contacting said host cell with a conditionally replicating viral vector of claim 14, wherein said viral vector further comprises the gene of interest and is capable of expressing the gene in said host cell, and a helper; and (b) expressing in said host cell the gene of interest.

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51. A method of expressing a gene of interest in a host cell, which method comprises:

(a) contacting said host cell with a conditionally replicating viral vector of claim 19, wherein said viral vector further comprises the gene of interest and is capable of expressing the gene in said host cell, and a helper; and (b) expressing in said host cell the gene of interest.

52. A method of detecting interaction between a drug/factor and a protein, which method comprises:

(a) contacting the drug/factor with a host cell of claim 28, in which the vector encodes and expresses a protein; and

(b) detecting interaction between the drug/factor and the mutant protein.

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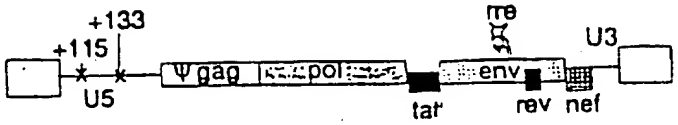
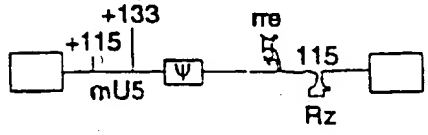


Figure 1A



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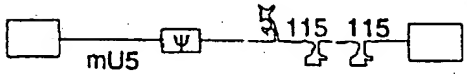

Figure 1C

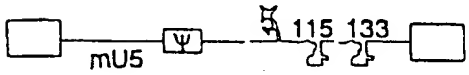

Figure 1D

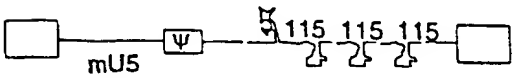

Figure 1E

BNSDOCID: <WO 9720060A1 I >

```
A   +105 GTGTGCCCGTCTG +117
B        .........AC...

A   +118 TTGTGTGACTCTG +130
B        .............

A   +131 GTAACTAGAGATC +143
B        .C.G.......A.
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Figure 2

INSDOCID: <WO___9720060A1_I_>

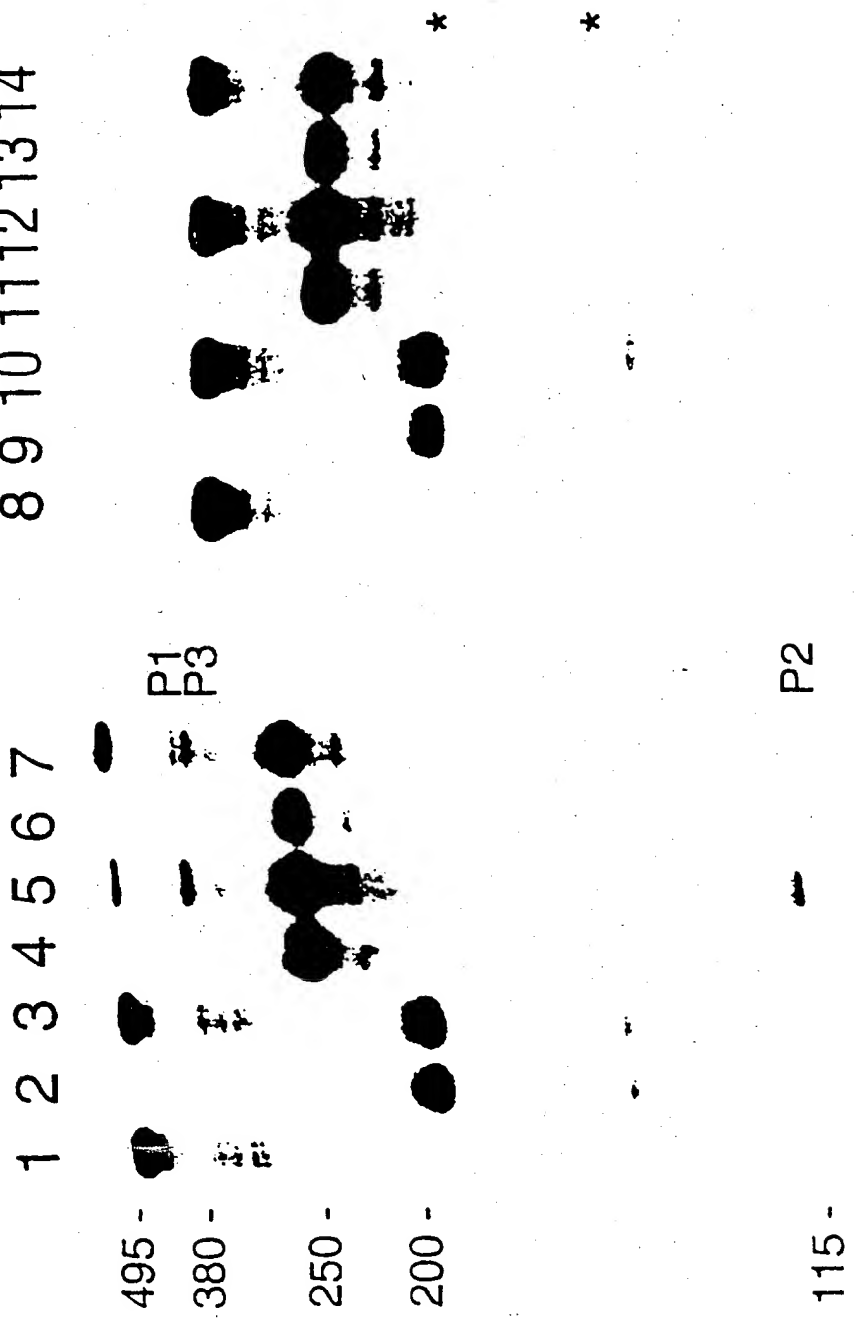

Figure 3

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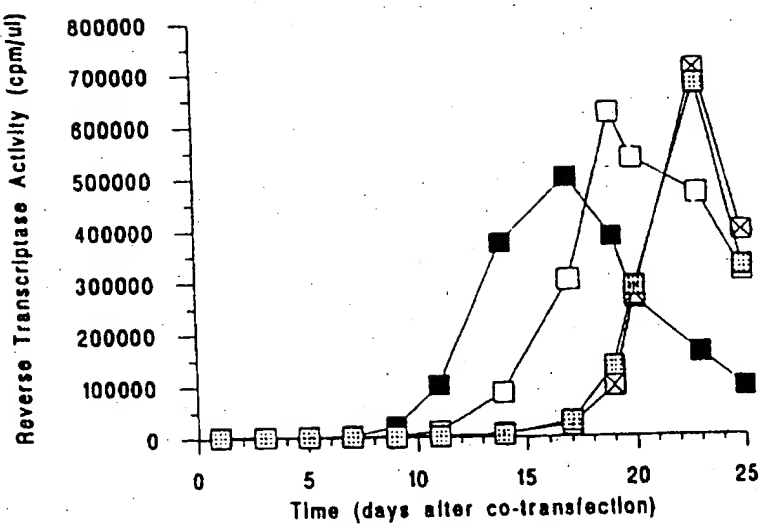

Figure 4

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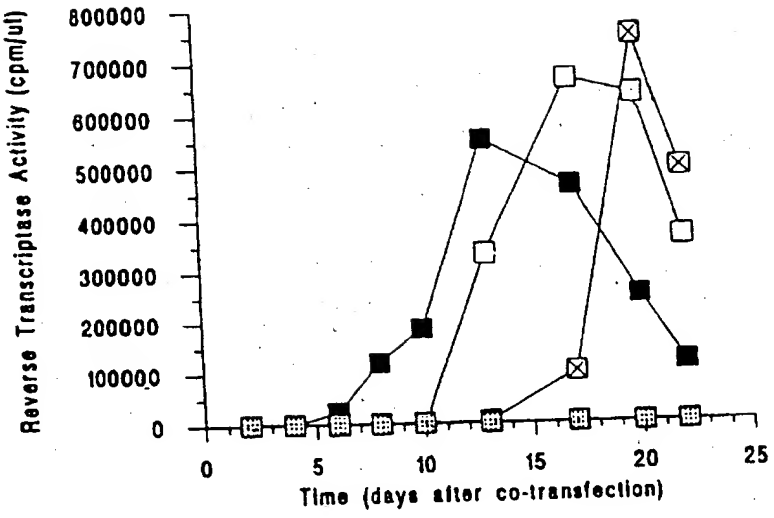

Figure 5

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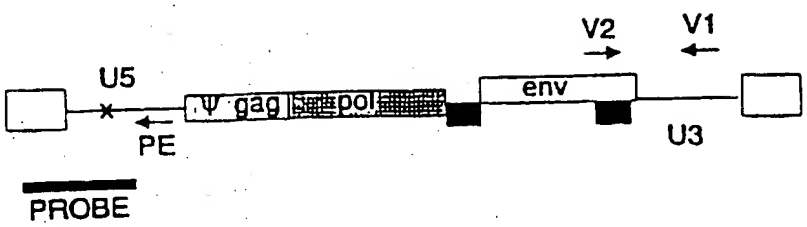

Figure 6A

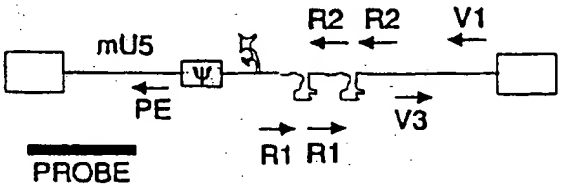

Figure 6B

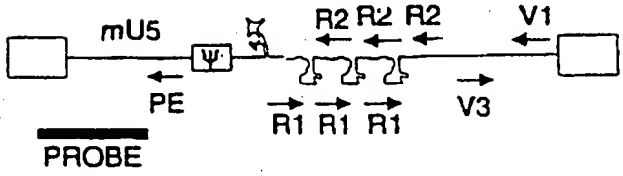

Figure 6C

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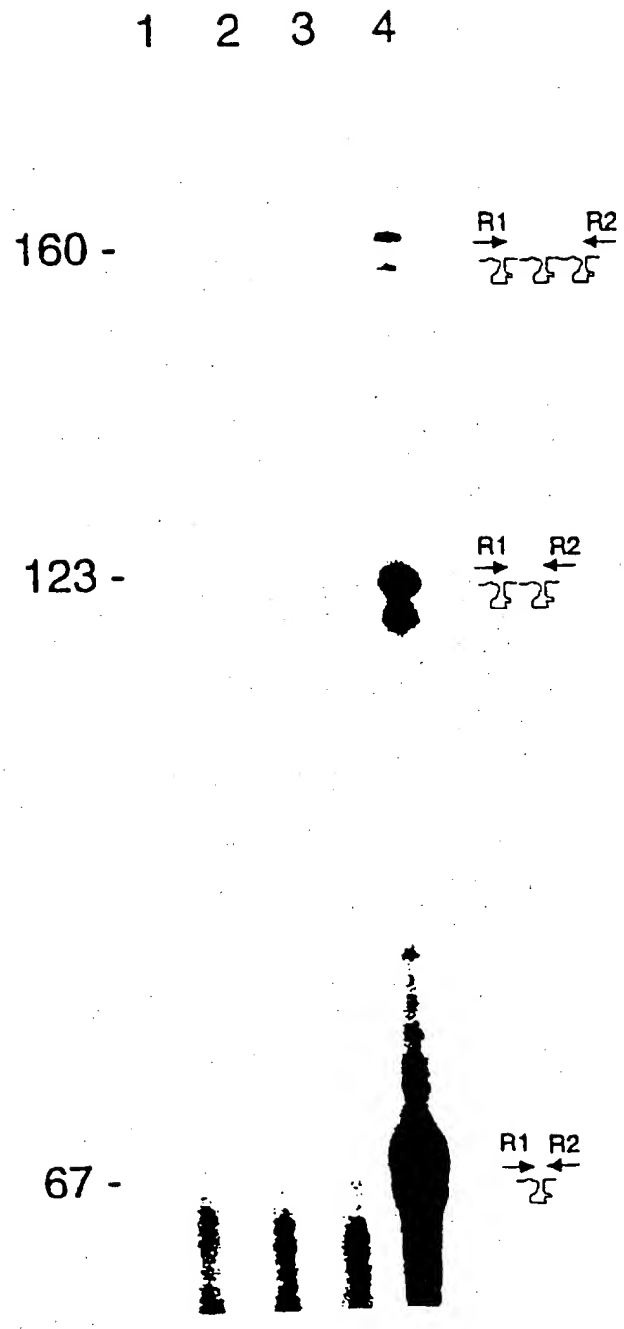

Figure 7

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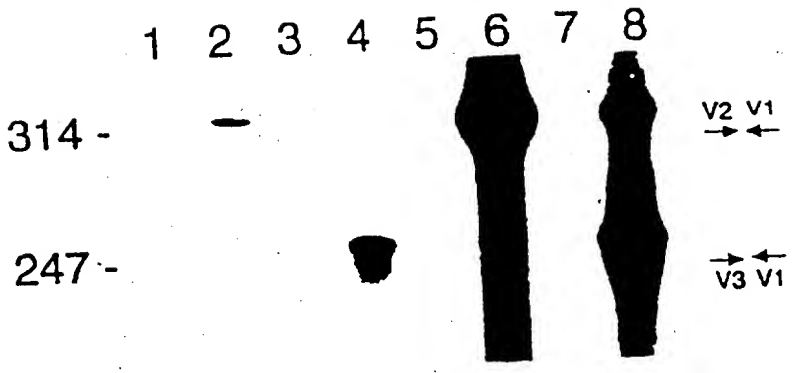

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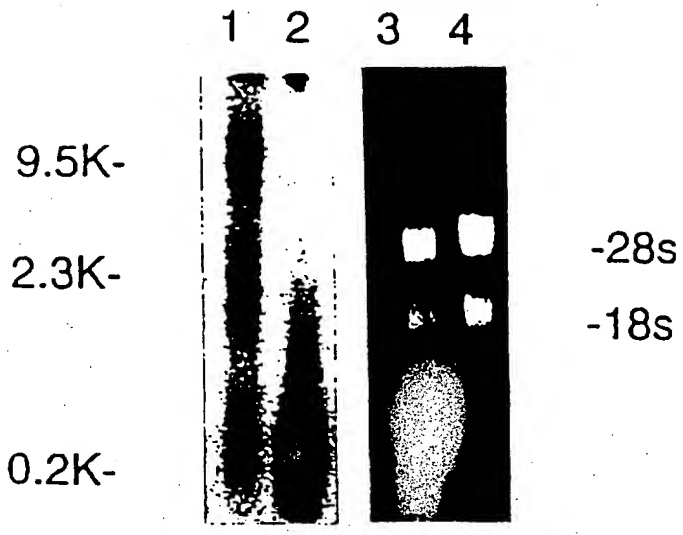

Figure 9

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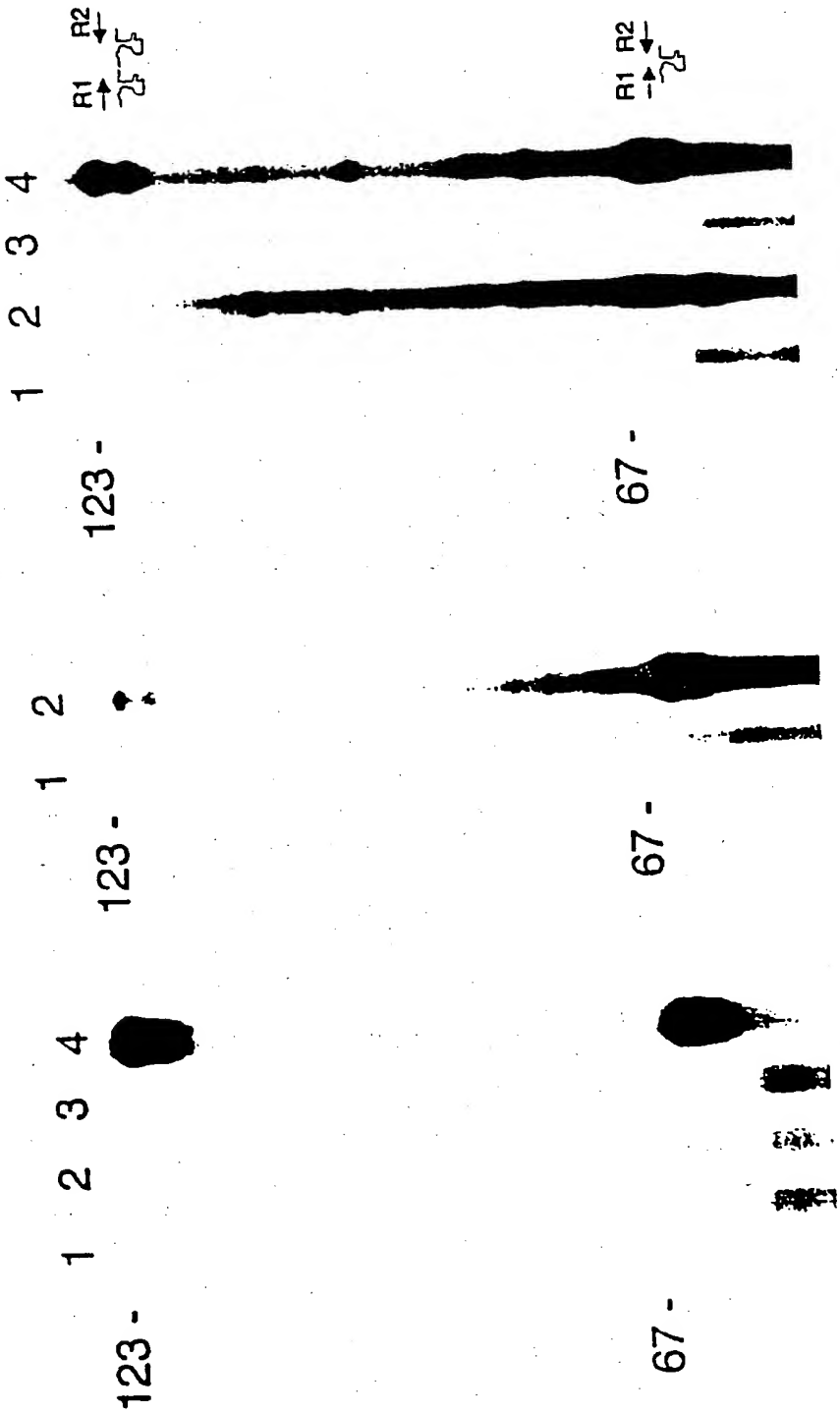

Figure 10C

Figure 10B

Figure 10A

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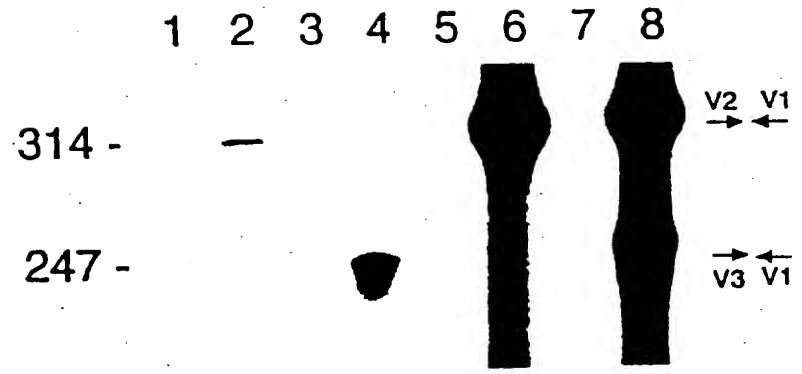

Figure 11

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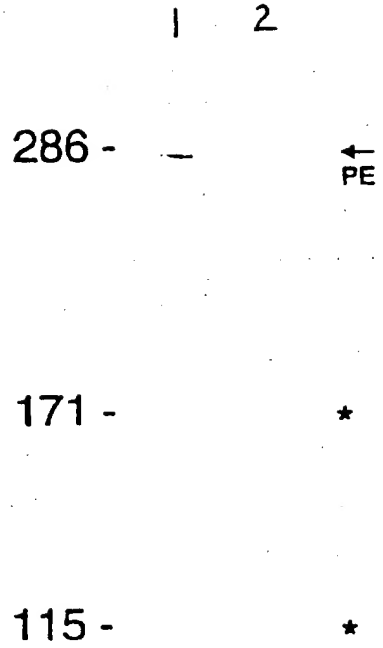

Figure 12

INSDOCID: <WO___9720060A1_I >

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/18997

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 C12N9/00 A61K48/00 C12N5/10 C12N15/49

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9551 Derwent Publications Ltd., London, GB; Class B04, AN 95-404121 XP002028428 & WO 95 30755 A (HISAMITSU PHARMACEUTICAL CO LTD) , 16 November 1995 see abstract ---	1-5,13, 14,16, 19,21, 25-30, 32,34, 36, 38-41, 43-52
X	EP 0 612 844 A (ORTHO PHARMACEUTICAL CORPORATION) 31 August 1994 see page 12, right-hand column - page 13 --- -/--	1-5,13, 14,16, 19,21, 25-30, 34,36, 38-41, 43,46-52

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 1997	08.04.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

BNSDOCID: <WO 9720060A1_I_>

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PC1/US 96/18997

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUMAN GENE THERAPY, vol. 5, no. 8, August 1994, pages 927-939, XP000647743 B. DROPULIC AND K-T. JEANG: "Gene therapy for Human Immunodeficiency Virus infection: Genetic antiviral strategies and targets for intervention" see page 928 ---	1-52
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, no. 20, 1 October 1996, WASHINGTON US, pages 11103-11108, XP002028427 B. DROPULIC ET AL.: "A conditionally replicating HIV-1 vector interferes with wild-type HIV-1 replication and spread" see the whole document -----	1-52

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INSDOCID: <WO___9720060A1_I_>

INTERNATIONAL SEARCH REPORT

'ernational application No.
PCT/US 96/ 18997

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 38-47
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: In so far these claims are directed to an in vivo method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

BNSDOCID: <WO__9720060A1 I_>

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ›nal Application No
PCI/US 96/18997

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0612844 A	31-08-94	AU 5639494 A	01-09-94
		CA 2116253 A	26-08-94
		FI 940867 A	26-08-94
		JP 6335392 A	06-12-94
		NO 940624 A	26-08-94
		ZA 9401287 A	24-08-95

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